J Vet Diagn Invest. 2000 Jan;12(1):28-32. Related Articles, Links

Improvement of western blot test specificity for detecting equine serum antibodies to Sarcocystis neurona.

Rossano MG, Mansfield LS, Kaneene JB, Murphy AJ, Brown CM, Schott HC 2nd, Fox JC.

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Equine protozoal myeloencephalitis (EPM) is a neurological disease of horses and ponies caused by the apicomplexan protozoan parasite Sarcocystis neurona. The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and to assess the ability of bovine antibodies to Sarcocystis cruzi to act as a blocking agent to minimize false-positive results in the western blot test for S. neurona. Sarcocystis neurona merozoites harvested from equine dermal cell culture were heat denatured, and the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient gel. Separated proteins were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6 horses with S. neurona infections (confirmed by culture from neural tissue) and 57 horses without infections (horses from the Eastern Hemisphere, where S. neurona does not exist) were tested by Western blot. Horses from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-kD bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the equine samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16-kD bands ($P<0.001$, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking proteins not specific to S. neurona and using reactivity to the 30- and 16-kD bands as the criterion for a positive test.

J Parasitol. 2000 Feb;86(1):25-32. Related Articles, Links

Comparative development and merozoite production of two isolates of Sarcocystis neurona and Sarcocystis falcatula in cultured cells.

Speer CA, Dubey JP, Mattson DE.

Department of Veterinary Molecular Biology, Montana State University, Bozeman 59717-3610, USA.

The development and merozoite production of Sarcocystis falcatula and 2 isolates (SN6 and SN2) of Sarcocystis neurona were studied in various cultured cell lines inoculated with culture-derived merozoites. All 3 parasites underwent multiple cycles of schizogony in VERO cells, bovine monocytes (M617 cells), and bovine pulmonary artery endothelial cells (CPA). Sarcocystis neurona strains SN6 and SN2 formed schizonts in rat myoblasts (L6) but not in quail myoblasts (QM7); S. falcatula formed schizonts in QM7 cells but not in L6 cells. Merozoites did not develop to sarcocysts in the myoblast cells lines. During a 47-day culture period in VERO cells, SN6 produced substantially more merozoites than did SN2 or S. falcatula. M617 cells produced substantially more merozoites of SN6 than did VERO or CPA cells. During a 17-day culture period of SN6, M617 cells produced mean totals of 4.7 x 10(8) merozoites, VERO cells produced 1.9 x 10(8) merozoites, and CPA cells produced 5.9 x 10(7) merozoites. At 4-12 days after inoculation of cultured cells with SN6, M617 cells cultured in the presence of 10% fetal bovine serum (FBS) produced a mean merozoite total of 5.1 x 10(8) compared to 3.6 x 10(8) for culture medium containing 1% FBS.

PMID: 10701559 [PubMed - indexed for MEDLINE]

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END OF SEARCH HISTORY

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DOCUMENT-IDENTIFIER: US 20040044055 A1
TITLE: N-alkoxlyalkyl-substituted benzimidazoles and the use thereof as an agent against parastic protozoans

Summary of Invention Paragraph:
[0068] Apicomplexa (Sporozoa) such as Eimeridae, for example, Eimeria acervulina, E. adenoides, E. alabahmensis, E. anatis, E. anseris, E. arloingi, E. ashata, E. auburnensis, E. bovis, E. brunetti, E. canis, E. chinchillae, E. clupearum, E. columbae, E. contorta, E. crandalis, E. debliecki, E. dispersa, E. ellipsoidales, E. falciformis, E. faurei, E. flavescens, E. gallopavonis, E. hagani, E. intestinalis, E. iroquoina, E. irresidua, E. labbeana, E. leucarti, E. magna, E. maxima, E. media, E. meleagridis, E. meleagrimitis, E. mitis, E. necatrix, E. ninakohlyakimovae, E. ovis, E. parva, E. pavonis, E. perforans, E. phasani, E. piriformis, E. praecox, E. residua, E. scabra, E. spec., E. stiedai, E. suis, E. tenella, E. truncata, E. truttae, E. zuernii, Globidium spec., Isospora belli, I. canis, I. felis, I. ohioensis, I. rivolta, I. spec., I. suis, Neospara caninum, Neospora hughesi; Cystisospora spec., Cryptosporidium spec. such as Toxoplasmadidae, for example, Toxoplasma gondii, such as Sarcocystidae, for example, Sarcocystis bovicanis, S. bovibominis, S. neurona, S. ovicanis, S. ovifelis, S. spec., S. suihominis such as Leucozoidae, for example, Leucozytozoon simondi, such as Plasmodiidae, for example, Plasmodium berghei, P. falciparum, P. malariae, P. ovale, P. vivax, P. spec., such as Piroplasmea, for example, Babesia argentina, B. bovis, B. canis, B. spec., Theileria parva, Theileria spec., such as Adeleina, for example, Hepatozoon canis, H. spec.

DOCUMENT-IDENTIFIER: US 20030032677 A1
TITLE: Targeted oxidative therapeutic formulation

Abstract Paragraph:
Pharmaceutical formulation, its method of preparation, and its use. The pharmaceutical formulation contains peroxidic species or reaction products resulting from oxidation of an alkene, such as geraniol, by an oxygen-containing oxidizing agent, such as ozone; a penetrating solvent, such as dimethyl sulfoxide; a dye containing a chelated metal, such as hematoporphyrin; and an aromatic redox compound, such as benzoquinone. The pharmaceutical formulation is used to treat horses infected with Sarcocystis protozoal infections.

Summary of Invention Paragraph:
[0055] The pharmaceutical formulation of this invention was utilized to eliminate Sarcocystis protozoal (Sarcocystis neurona) infections in horses afflicted with Equine Protozoal Myeloencephalitis ("EPM"), which is a costly, debilitating, and eventually fatal neurological disease. Examples Two and Three detail the retrospective non-randomized study. The trial evaluated three hundred forty-four consecutive horses diagnosed and treated for EPM.

Summary of Invention Paragraph:
[0056] EPM is currently the most common neurological condition afflicting horses in North and South America. EPM is usually caused by infection of the spinal and cranial nerve tracts with the parasite, Sarcocystis Neurona. EPM has been reported to produce numerous syndromes of central nervous system dysfunction. S. Neurona has the feral opossum (Didelphis virginiana) as its primary host. North and South American horses appear to be an aberrant host for EPM, because the merozoites continually divide in the central nervous system, without encysting. Horses with EPM most commonly have abnormalities of gait, but they also may present with other signs of brain disease, including optic nerve blindness. The disease ranges in severity from mild lameness to sudden recumbence, and clinical signs are progressive.

US-PAT-NO: 6448252
DOCUMENT-IDENTIFIER: US 6448252 B1

TITLE: Treatment of equine protozoal myeloencephalitis

DATE-ISSUED: September 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Fenger; Clara K.	Lexington	KY		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
IDEXX Pharmaceuticals, Inc.	Greensboro	NC			02

APPL-NO: 09/ 685943 [PALM]
DATE FILED: October 10, 2000

PARENT-CASE:
This a is a continuation of U.S. application Ser. No. 09/069,956 filed Apr. 30, 1998 now U.S. Pat. No. 6,255,308, hereby incorporated by reference in its entirety, which is a continuation of U.S application Ser. No 08/683,507 filed Jul. 17, 1996, now U.S. Pat. No. 5,747,476.

INT-CL: [07] A61 K 31/505, A61 K 31/18

US-CL-ISSUED: 514/256; 514/258, 514/275, 514/601
US-CL-CURRENT: 514/256; 514/251, 514/275, 514/601

FIELD-OF-SEARCH: 514/256, 514/275, 514/601, 514/258

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected

What is claimed is:

1. A method of therapeutically treating a diseased mammal suffering from a parasitic neurologic or abortigenic disease that is susceptible to being treated with a praziquantel compound, comprising administering to the mammal a composition of a pharmaceutically effective amount of praziquantel or a derivative thereof and a pharmaceutically acceptable carrier.

2. The method of claim 1 wherein the disease is caused by a coccidia.

3. The method of claim 2 wherein the coccidia is a member of the group consisting of Sarcocystis spp, Neospora spp, Toxoplasma spp and Isospora spp.

4. The method of claim 3 wherein the Sarcocystis spp is Sarcocystis neurona, the Neospora spp is Neospora caninum or Neospora hugesi, the Toxoplasma spp is Toxoplasma gondii and the Isospora spp is Isospora suis.

5. The method of claim 4 wherein the Sarcocystis neurona is the causative agent of Equine Protozoal Myeloencephalitis.

6. The method of claim 3 wherein the Neospora caninum is the causative agent of bovine or canine Neosporosis.

7. The method of claim 3 wherein the Neospora hugesi is the causative agent of Equine Protozoal Myeloencephalitis.

8. The method of claim 3 wherein the Toxoplasma gondii is the causative agent of Toxoplasma-associated abortion in mammals.

9. A method for treating a diseased mammal suffering from a parasitic neurologic or abortigenic disease that is susceptible to being treated with a praziquantel compound, comprising administering to the mammal a neurologically-effective or placentally-effective amount of praziquantel or a derivative thereof and a pharmaceutically acceptable carrier.

10. The method of metaphylactically treating mammals infected with a parasite that is a causative agent for a neurologic or abortigenic disease that is susceptible to being treated with a praziquantel compound, comprising administering thereto a methaphylactically-effective regimen of a composition of a pharmaceutically effective amount of the praziquantel compound or a derivative thereof and a pharmaceutically-acceptable carrier.

11. The method of claim 1 wherein the composition is administered in two or more intermittent doses.

12. The method of claim 1 wherein the composition is administered in a dose of about 1.0 and 100 mg/Kg.

13. The method of claim 2 wherein the composition is administered in a dose of about 1.0 and 50 mg/Kg.

14. The method of claim 1 wherein the composition is administered in a single high dose of greater than 50 mg/Kg.

EQUINE PROTOZOAL MYELOENCEPHALITIS VACCINE

VACCIN CONTRE LA MYELOENCEPHALITE PROTOZOAIRE DU CHEVAL

Patent Applicant/Assignee:
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Patent and Priority Information (Country, Number, Date):
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Application: WO 2001US40527 20010413 (PCT/WO US0140527)
Priority Application: US 2000199435 20000425; US 2001278695 20010326

Designated States:
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E7     1039    3  SARCOCYSTOSIS
E8       36       SARCOCYSTOSIS --BLOOD --BL
E9        9       SARCOCYSTOSIS --CEREBROSPINAL FLUID --CF
E10      56       SARCOCYSTOSIS --COMPLICATIONS --CO
E11       4       SARCOCYSTOSIS --CONGENITAL --CN
E12     108       SARCOCYSTOSIS --DIAGNOSIS --DI

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      S2     192  SARCOCYSTIS? AND NEURONA?
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Set     Items   Description
S1         15   NEOSPORA? AND HUGHESI?
S2        192   SARCOCYSTIS? AND NEURONA?
? s s1 and s2
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             192  S2
      S3       7  S1 AND S2
? t s3/9/all
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12685232 PMID: 10608440

Prevalence of antibodies to Neospora sp. in horses from Alabama and characterisation of an isolate recovered from a naturally infected horse [corrected]

Cheadle M A; Lindsay D S; Rowe S; Dykstra C C; Williams M A; Spencer J A; Toivio-Kinnucan M A; Lenz S D; Newton J C; Rolsma M D; Blagburn B L

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849, USA.

International journal for parasitology (ENGLAND) Oct 1999, 29 (10) p1537-43, ISSN 0020-7519 Journal Code: 0314024

Publishing Model Print; Erratum in Int J Parasitol 2000 Apr 24;30(5) 677

Document type: Case Reports; Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

An IFAT was used to determine the prevalence of **Neospora** -specific IgG antibodies in serum from Alabama horses. Serum samples (n = 536) were from asymptomatic horses routinely submitted for equine infectious anaemia virus infection testing. We also subjected a 13-year-old horse with CNS disease to necropsy examination for isolation and in vitro cultivation of protozoal organisms. In antemortem tests, this horse was positive for antibodies to **Neospora** sp. in the IFAT and western immunoblot. Results of the prevalence survey indicated that IgG antibodies to **Neospora** were present in 62 (11.5%) of the 536 serum samples. Endpoint titres for the positive samples were 1:50 (35/6.5%), 1:100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). Tachyzoites were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the horse with CNS disease. Tachyzoites reacted with known N. caninum-positive serum from horses, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or equine anti- **Sarcocystis neurona** serum. Ultrastructural features of tachyzoites and results of comparison of tachyzoite immunodominant proteins revealed that they were identical to those of N. **hughesi** , a species described recently from a naturally infected horse. The isolate recovered from the naturally infected horse in the present study (designated NA1) is thought to be an isolate of N. **hughesi** , although confirmation of this awaits additional molecular characterisation. These results provide some additional evidence that N. **hughesi** is a valid species and that **Neospora** infections in horses may occur in widely separated geographic regions of the United States.

Tags: Female; Research Support, Non-U.S. Gov't

Descriptors: *Antibodies, Protozoan--blood--BL; *Coccidiosis--veterinary --VE; *Horse Diseases--epidemiology--EP; * **Neospora** --immunology--IM; * **Neospora** --isolation and purification--IP; Animals; Antibodies, Protozoan --immunology--IM; Cattle; Coccidiosis--epidemiology--EP; Coccidiosis --parasitology--PS; Dogs; Fluorescent Antibody Technique, Indirect; Horse Diseases--parasitology--PS; Horses; Mice; Myelitis--parasitology--PS; Myelitis--veterinary--VE; **Neospora** --ultrastructure--UL; Prevalence; Spinal Cord--parasitology--PS

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20000120

Record Date Completed: 20000120

12685232 PMID: 10608440

Prevalence of antibodies to Neospora **sp. in horses from Alabama and characterisation of an isolate recovered from a naturally infected horse [corrected]**

Cheadle M A; Lindsay D S; Rowe S; Dykstra C C; Williams M A; Spencer J A; Toivio-Kinnucan M A; Lenz S D; Newton J C; Rolsma M D; Blagburn B L

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849, USA.

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Document type: Case Reports; Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

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Subfile: INDEX MEDICUS

An IFAT was used to determine the prevalence of **Neospora** -specific IgG antibodies in serum from Alabama horses. Serum samples (n = 536) were from asymptomatic horses routinely submitted for equine infectious anaemia virus infection testing. We also subjected a 13-year-old horse with CNS disease to necropsy examination for isolation and in vitro cultivation of protozoal organisms. In antemortem tests, this horse was positive for antibodies to **Neospora** sp. in the IFAT and western immunoblot. Results of the prevalence survey indicated that IgG antibodies to **Neospora** were present in 62 (11.5%) of the 536 serum samples. Endpoint titres for the positive samples were 1:50 (35/6.5%), 1:100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). Tachyzoites were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the horse with CNS disease. Tachyzoites reacted with known N. caninum-positive serum from horses, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or equine anti- **Sarcocystis neurona** serum. Ultrastructural features of tachyzoites and results of comparison of tachyzoite immunodominant proteins revealed that they were identical to those of N. **hughesi** , a species described recently from a naturally infected horse. The isolate recovered from the naturally infected horse in the present study (designated NA1) is thought to be an isolate of N. **hughesi** , although confirmation of this awaits additional molecular characterisation. These results provide some additional evidence that N. **hughesi** is a valid species and that **Neospora** infections in horses may occur in widely separated geographic regions of the United States.

Tags: Female; Research Support, Non-U.S. Gov't

Descriptors: *Antibodies, Protozoan--blood--BL; *Coccidiosis--veterinary --VE; *Horse Diseases--epidemiology--EP; * **Neospora** --immunology--IM; * **Neospora** --isolation and purification--IP; Animals; Antibodies, Protozoan --immunology--IM; Cattle; Coccidiosis--epidemiology--EP; Coccidiosis --parasitology--PS; Dogs; Fluorescent Antibody Technique, Indirect; Horse Diseases--parasitology--PS; Horses; Mice; Myelitis--parasitology--PS; Myelitis--veterinary--VE; **Neospora** --ultrastructure--UL; Prevalence; Spinal Cord--parasitology--PS

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20000120

Record Date Completed: 20000120

? t s4/3/18

4/3/18 (Item 3 from file: 399)

135343283 CA: 135(24)343283y PATENT

Equine protozoal myeloencephalitis vaccine

INVENTOR(AUTHOR): Bigbie, Rocky Barry; Ng, Terry Kaleung; Whalen, Joseph Wilson, Jr.

LOCATION: USA

ASSIGNEE: American Home Products Corporation

PATENT: PCT International ; WO 200180885 A2 DATE: 20011101

APPLICATION: WO 2001US40527 (20010413) *US PV199435 (20000425) *US PV278695 (20010326)

PAGES: 31 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: A61K-039/00A

DESIGNATED COUNTRIES: AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; BZ; CA; CH; CN; CO; CR; CU; CZ; DE; DK; DM; DZ; EE; ES; FI; GB; GD; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA; MD; MG; MK; MN; MW; MX; MZ; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; TZ; UA; UG; UZ; VN; YU; ZA; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM DESIGNATED REGIONAL: GH; GM; KE; LS; MW; MZ; SD; SL; SZ; TZ; UG ; ZW; AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; TR; BF; BJ; CF; CG; CI; CM; GA; GN; GW; ML;

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merozoite? or tachyzoite?)

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            1    35: Dissertation Abs Online_1861-2005/May
            3    50: CAB Abstracts_1972-2005/May
            3    71: ELSEVIER BIOBASE_1994-2005/Jun W1
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      Examined  50 files
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            2   162: Global Health_1983-2005/May
            1   185: Zoological Record Online(R)_1978-2005/Jun
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N37            0    51: Food Sci.&Tech.Abs_1969-2005/Jun W2
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             155  HUGHESI
           26005  IMMUNIZE
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                  INJECT? OR MEROZOITE? OR TACHYZOITE?)
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Cornell Vet. 1992 Jan;82(1):41-52. Related Articles, Links

Erratum in:
- Cornell Vet 1992 Apr;82(2):115.

Characterization of Sarcocystis neurona from a thoroughbred with equine protozoal myeloencephalitis.

Bowman DD, Cummings JF, Davis SW, deLahunta A, Dubey JP, Suter MM, Rowland PH, Conner DL.

Department of Microbiology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853.

Morphological information is presented for syntype material of the etiologic agent of equine protozoal myeloencephalitis, Sarcocystis neurona. A clinical description of the horse from which the organism was isolated and the methodology used to immunosuppress the horse in an attempt to increase parasite numbers are also given. The description includes microscopic details observed both with light and transmission electron microscopy. Mainly stages from tissue are illustrated, but information is also presented on the development of the organism after inoculation onto monolayers of bovine monocytes. It is believed that the large numbers of organisms observed in this horse were due to its having not received prior treatment with trimethoprimsulphonamide and the large amounts of corticosteroids that were administered in order to facilitate isolation of the pathogen.

Publication Types:
- Case Reports

Neospora caninum-associated equine protozoal myeloencephalitis

A. N. Hamir[a,*], S. J. Tornquist[a], T. C. Gerros[a], M. J. Topper[b] and J. P. Dubey[c]

[a] College of Veterinary Medicine, Oregon State University Corvallis, OR 97331 USA

[b] Division of Pathology, Walter Reed Army Institute of Research Washington, DC 20307 USA

[c] Parasite Biology and Epidemiology Laboratory,Livestock and Poultry Sciences Institute, ARS, USDA, BARC-East Beltsville, MD 20705 USA

Received 19 February 1998; accepted 15 May 1998. Available online 21 November 1998.

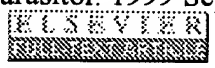
Abstract

Equine protozoal myeloencephalitis (EPM) was clinically diagnosed in a 20-year-old horse with severe ataxia. The cerebrospinal fluid was positive for *Sarcocystis neurona* antibodies by western blot. The horse was administered corticosteroids to facilitate in vitro culture of *S. neurona* from its spinal cord following necropsy. Microscopic lesions of EPM were present in the brain and in the spinal cord, including multifocal inflammatory cellular infiltrates and several large groups of protozoa. Immunohistochemical, and light and electron microscopic examinations revealed that the protozoa were *Neospora caninum* and not *S. neurona*. The protozoa divided by endodyogeny, tachyzoites had rhoptries, and organisms reacted specifically to *N. caninum* antibodies. Veterinarians should be aware of increasing diagnosis of *N. caninum* as another etiological agent responsible for the lesions of EPM.

Author Keywords: Neosporosis; Horse; Myeloencephalitis; *Neospora caninum*

Index Terms: neospora caninum; encephalomyelitis; horse disease

Vet Parasitol. 1999 Sep 15;86(1):59-62. Related Articles, Links



Prevalence of antibodies to Sarcocystis neurona, Toxoplasma gondii and Neospora caninum in horses from Argentina.

Dubey JP, Venturini MC, Venturini L, McKinney J, Pecoraro M.

Parasite Biology and Epidemiology Laboratory, United States Department of Agriculture, Agricultural Research Service, Livestock and Poultry Sciences Institute, Beltsville, MD 20705-2350, USA. jdubey@lpsi.barc.usda.gov

Sera from 76 horses from Argentina were examined for antibodies to Sarcocystis neurona, Toxoplasma gondii and Neospora caninum. Antibodies to S. neurona were found in 27 (35.5%) of 76 horses using immunoblots with culture derived merozoites as antigen. Antibodies to T. gondii were found in 10 (13.1%) of 76 horses by using the modified agglutination test with formalin-fixed tachyzoites and mercaptoethanol; titers were 1:25 (two horses), 1:50 (six horses), 1:100 (two horses), and 1:200 (one horse). Antibodies to N. caninum were not found in any of the 76 horses by the use of N. caninum agglutination test. This is the first report of S. neurona infection in horses in Argentina.

PMID: 10489203 [PubMed - indexed for MEDLINE]

J Am Vet Med Assoc. 1999 Oct 1;215(7):970-2. Related Articles, Links

Serologic prevalence of Sarcocystis neurona, Toxoplasma gondii, and Neospora caninum in horses in Brazil.

Dubey JP, Kerber CE, Granstrom DE.

Parasite Biology and Epidemiology Laboratory, United States Department of Agriculture, Beltsville Agricultural Research Center, MD 20705-2350, USA.

OBJECTIVE: To determine serologic prevalence of Sarcocystis neurona, Toxoplasma gondii, and Neospora caninum in horses in Brazil. DESIGN: Prevalence survey. ANIMALS: 101 Thoroughbreds in Brazil. PROCEDURE: Blood samples were obtained from horses and tested for serum antibodies against S neurona by use of an immunoblot procedure with culture-derived S neurona merozoites as antigen, and for serum antibodies against T gondii and N caninum by use of a modified agglutination test with formalin-preserved tachyzoites and mercaptoethanol. RESULTS: Antibodies against S neurona and T gondii were detected in 36 and 16 of 101 horses, respectively. Cross-reactivity between antibodies against T gondii and S neurona was not detected. Antibodies against N caninum were not detected in any samples. CONCLUSIONS AND CLINICAL RELEVANCE: The high prevalence of antibodies against S neurona detected in clinically normal horses emphasizes the importance of examining CSF for antibodies when establishing a diagnosis of equine protozoal myeloencephalitis.

PMID: 10511862 [PubMed - indexed for MEDLINE]

J Parasitol. 1999 Oct;85(5):979-81. Related Articles, Links

Simplified technique for isolation, excystation, and culture of Sarcocystis species from opossums.

Murphy AJ, Mansfield LS.

Animal Health Diagnostic Laboratory, Michigan State University, East Lansing 48824, USA.

Sarcocystis neurona is a protozoan parasite that causes a neurological disease in horses called equine protozoal myeloencephalitis. The route of transmission is speculated to be by fecal-oral transfer of sporocysts shed from opossums. Controversy exists regarding both the natural life cycle for this parasite as well as the species identity of opossum Sarcocystis. To provide stage-specific material for species comparison, 27 opossums from southern Michigan were screened for Sarcocystis spp. sporocysts. Seven opossums were positive for Sarcocystis sporocysts by fecal flotation. A simplified, effective technique for isolation, excystation, and culture of opossum Sarcocystis sp. from mucosal scrapings was developed. All 7 Sarcocystis sp. isolates were successfully cultured to grow long term in equine dermal cells to the merozoite stage. Merozoites were observed between 5 and 15 days after inoculation. In conclusion, opossums shed Sarcocystis sp. sporocysts that may be manipulated to excyst and grow in vitro in equine dermal cell lines to the merozoite stage using the simplified technique described.

PMID: 10577742 [PubMed - indexed for MEDLINE]

J Eukaryot Microbiol. 1999 Sep-Oct;46(5):500-6. Related Articles, Links

Characterization of a Sarcocystis neurona isolate (SN6) from a naturally infected horse from Oregon.

Dubey JP, Mattson DE, Speer CA, Baker RJ, Mulrooney DM, Tornquist SJ, Hamir AN, Gerros TC.

United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Maryland 20705-2350, USA. Jdubey@lpsi.barc.usda.gov

An isolate of Sarcocystis neurona (SN6) was obtained from the spinal cord of a horse from Oregon with neurologic signs. The parasite was isolated in cultures of bovine monocytes and equine spleen cells. The parasite divided by endopolygeny and completed at least one asexual cycle in cell cultures in three days. Two gamma interferon knockout mice inoculated with cell culture-derived merozoites became ill 35 d later and S. neurona schizonts and merozoites were found in encephalitic lesions. The parasite in tissue sections of mice reacted with S. neurona-specific antibodies and S. neurona was reisolated from the brain of knockout mice.

Publication Types:
- Case Reports

PMID: 10519218 [PubMed - indexed for MEDLINE]

1. 6489148. 12 May 00; 03 Dec 02. Immunoassay for equine protozoal myeloencephalitis in horses. Mansfield; Linda S., et al. 435/183; 435/34 435/7.1 435/7.2 435/7.22 436/518 530/388.6. C12N009/00 G01N033/55 G01N033/53 G01N033/567 C12Q001/04.

2. 6344337. 18 Feb 00; 05 Feb 02. Antigen test to detect equine protozoal myeloencephalitis in horse serum and cerebrospinal fluid. Mansfield; Linda S., et al. 435/7.2; 435/34 530/388.6. G01N033/53.

3. 6153394. 18 Sep 98; 28 Nov 00. Immunoassay for equine protozoal myeloencephalitis in horses. Mansfield; Linda S., et al. 435/7.22; 435/7.1 436/518 436/523 436/528 436/543. G01N033/52 G01N033/569 G01N033/53 G01N033/543 G01N033/544.

4. WO 200115708A. Vaccinating equids against protozoal Sarcocystis neurona infections using unique antigens. MANSFIELD, L S, et al. A61K031/70 A61K038/00 A61K039/395 C07H021/02 C07K001/00 C07K014/00 C12N005/00 C12N015/00 C12P021/06.

5. US 6344337B. Detection of Sarcocystis neurona, which causes equine protozoal myeloencephalitis, in horse serum and cerebrospinal fluid comprises identifying a specific antibody-antigen complex via an immunoassay. MANSFIELD, L S, et al. C07K016/00 C07K017/00 C07K017/14 C12P021/08 G01N033/53 G01N033/537 G01N033/543 G01N033/553.

6. US 6153394A. Immunoassay for equine protozoal myeloencephalitis in horses uses specific antibodies to proteins derived from Sarcocystis neurona. MANSFIELD, L S, et al. C12N009/00 C12Q001/04 G01N033/52 G01N033/53 G01N033/531 G01N033/543 G01N033/544 G01N033/55 G01N033/567 G01N033/569.

Biologics has renewed the conditional licence for Fort Dodge Animal Health's **Sarcocystis neurona** , killed protozoa **vaccine** for one year. The **vaccine** is intended to help prevent **equine** protozoal myeloencephalitis (**EPM**) by aiding in the prevention of new infections by S . **neurona** , the organism that causes **EPM** , the company says. The renewal was given as a result of the USDA's review of information supplied by Fort Dodge describing its progress toward fulfilling the requirements for a full licence. The data submitted included: a report describing challenge model development experiments conducted and in progress; a preliminary report describing studies of the **vaccine** 's ability to induce cell-mediated immune responses; a progress update concerning the planned multi-centre field performance study of the **vaccine** ; and a report updating the long-term safety of the **vaccine** in which field-trial horses were monitored following an annual booster administration. No adverse events were noted in the 392 horses.
ANIMAL PHARM - World Animal Health & Nutrition News FILED 29 October 2001

10/9/67 (Item 3 from file: 50)
DIALOG(R)File 50:CAB Abstracts

0008241943 CAB Accession Number: 20023102736

Seroprevalence of Neospora , Toxoplasma gondii and Sarcocystis **neurona antibodies in horses from Jeju island, South Korea.**

Gupta, G. D.; Lakritz, J.; Kim JaeHoon; Kim DaeYong; Kim JinKap; Marsh, A. E.

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Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Connaway Hall, 1600 East Rollins Dr., Columbia, MO 65211, USA.

Veterinary Parasitology vol. 106 (3): p.193-201

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Publisher: Elsevier Science B.V. Amsterdam, Netherlands

Language: English Record Type: Abstract

Document Type: Journal article

Parasite-specific antibody responses to Neospora spp. and Toxoplasma gondii **antigens** were detected using the indirect fluorescent antibody test (IFAT) and immunoblot analysis in 191 Thoroughbred horses (108 males and 83 females, 1.5-2 years old) from Jeju island, South Korea [date not given]. For comparison, a naturally infected Neospora hughesi horse and an experimentally inoculated T. gondii equid (pony) were used. In addition, all samples were tested for antibodies to **Sarcocystis** neurona by immunoblot analysis. A total of 191 serum samples from clinically normal horses were evaluated. Only 2% (4 out of 191) and 2.6% (5 out of 191) of the samples had showed reactivity at 1:100 using the IFAT for Neospora spp. and T. gondii , respectively. For T. gondii , two samples matched the **antigen** banding pattern of the positive control by immunoblot analysis. No sample was positive for N. hughesi by immunoblot analysis in this study. Overall, there was a 1% seroprevalence for T. gondii antibodies in the horses tested based on immunoblot analysis. The seroprevalence for S. **neurona** and N. hughesi antibodies was 0%. We concluded that these horses are either not routinely exposed to these parasites or the antibody titres are not sufficiently elevated to be detectable. It is most likely the

former explanation since Jeju island **equine** farms are isolated from the main land, and the horses were all less than 3 years of age. This naive population of horses could be useful when evaluating S. **neurona** serodiagnostic tests or evaluating potential S. **neurona** **vaccines** since exposure risks to S. **neurona** and closely related parasites are negligible.
42 ref.

DESCRIPTORS: disease prevalence; neosporosis; **sarcocystosis** ; seroprevalence; Thoroughbred; toxoplasmosis
IDENTIFIERS: Neospora hughesi
ORGANISM DESCRIPTORS: horses; Neospora; **Sarcocystis** neurona; Toxoplasma gondii
GEOGRAPHIC NAMES: Korea Republic
BROADER TERMS: Equus; Equidae; Perissodactyla; mammals; vertebrates; Chordata; animals; ungulates; East Asia; Asia; Developing Countries; Threshold Countries; OECD Countries; **Sarcocystidae** ; Eucoccidiorida; Apicomplexa; Protozoa; invertebrates; Neospora; **Sarcocystis** ; Toxoplasma
CABICODES: Protozoan, Helminth, Mollusc and Arthropod Parasites of Animals, (New March 2000) (LL822)

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? e neospora hughesi

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E2        0    1  NEOSPORA CANINUM
E3        0      *NEOSPORA HUGHESI
E4        1       NEOSPORACANINUM
E5        1       NEOSPORAL
E6        2       NEOSPORANS
E7       74       NEOSPORIN
E8        6       NEOSPOROSE
E9      219       NEOSPOROSIS
E10       1       NEOSPROSTONIA
E11       2       NEOSQUAMOCOLUMNAR
E12       1       NEOSQUAMOCOLUNMAR

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            839  NEOSPORA?
             16  HUGHESI?
      S1     15  NEOSPORA? AND HUGHESI?
? e sarcocystis neurona

Ref    Items   RT  Index-term
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E2       264       SARCOCYSTIS --ULTRASTRUCTURE --UL
E3         0      *SARCOCYSTIS NEURONA
E4         1       SARCOCYSTISFALCATULA
E5         1       SARCOCYSTIT
E6         1       SARCOCYSTOSES
E7      1039    3  SARCOCYSTOSIS
E8        36       SARCOCYSTOSIS --BLOOD --BL
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           1390  SARCOCYSTIS?
         124183  NEURONA?
      S2    192  SARCOCYSTIS? AND NEURONA?
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S2        192   SARCOCYSTIS? AND NEURONA?
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             15  S1
            192  S2
      S3      7  S1 AND S2
? t s3/9/all

 3/9/1
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17396919   PMID: 15715226
   Risk of transplacental transmission of Sarcocystis neurona and
 Neospora hughesi in California horses.
  Duarte Paulo C; Conrad Patricia A; Barr Bradd C; Wilson W David; Ferraro
Gregory L; Packham Andrea E; Carpenter Tim E; Gardner Ian A
  Department of Medicine and Epidemiology, School of Veterinary Medicine,
University of California, One Shields Avenue, Davis, California 95616, USA.
pdduarte@ucdavis.edu
  Journal of parasitology (United States)  Dec 2004,  90  (6)  p1345-51,
ISSN 0022-3395  Journal Code: 7803124
  Publishing Model Print
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: MEDLINE; Completed
  Subfile:  INDEX MEDICUS
  The study objective was to assess the risk of transplacental transmission
of Sarcocystis neurona and Neospora hughesi in foals from 4
California farms during 3 foaling seasons. Serum of presuckle foals and
serum and colostrum of periparturient mares were tested using indirect
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fluorescent antibody tests for S. **neurona** and N. **hughesi** . Serum antibody titers were < or =10 in 366 presuckle foals tested. There was no serologic or histologic evidence of either parasite in aborted fetuses or placentas examined. Positivity for S. **neurona** and N. **hughesi** in mares increased with age. Mares < or =9 yr that originated from Kentucky were 3.8 and 1.4 times more likely to be positive for S. **neurona** and N. **hughesi** , respectively, than mares from California. The strength of association between positivity to either parasite and state of birth decreased as age increased. Mares positive for S. **neurona** and N. **hughesi** were 2.2 and 1.7 times more likely, respectively, to have a previous abortion than negative mares, adjusted for age and state of birth. The annual mortality rate for mares was 4%. The annual incidence rate of equine protozoal myeloencephalitis was 0.2%. In conclusion, there was no detectable risk of transplacental transmission of S. **neurona** and N. **hughesi** . Prevalence of antibodies against both parasites in mares increased with age.

Tags: Female; Pregnancy; Research Support, Non-U.S. Gov't

Descriptors: *Coccidiosis--veterinary--VE; *Disease Transmission, Vertical--veterinary--VE; *Horse Diseases--transmission--TM; * **Neospora** --immunology--IM; *Pregnancy Complications, Parasitic; *Sarcocystosis --veterinary--VE; Abortion, Veterinary--epidemiology--EP; Abortion, Veterinary--parasitology--PS; Animals; Antibodies, Protozoan--blood--BL; California--epidemiology--EP; Coccidiosis--epidemiology--EP; Coccidiosis --transmission--TM; Cohort Studies; Colostrum--immunology--IM; Colostrum --parasitology--PS; Encephalomyelitis--epidemiology--EP; Encephalomyelitis --parasitology--PS; Encephalomyelitis--veterinary--VE; Fluorescent Antibody Technique, Indirect--veterinary--VE; Horse Diseases--epidemiology --EP; Horse Diseases--parasitology--PS; Horses; Incidence; Pregnancy; Pregnancy Complications, Parasitic--epidemiology--EP; Pregnancy Complications, Parasitic--parasitology--PS; Prevalence; Prospective Studies ; Risk Factors; **Sarcocystis** --immunology--IM; Sarcocystosis--epidemiology --EP; Sarcocystosis--transmission--TM

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20050217

Record Date Completed: 20050307

3/9/2

DIALOG(R)File 155:MEDLINE(R)

15462098 PMID: 15334837

Risk of postnatal exposure to Sarcocystis neurona **and** Neospora hughesi **in horses.**

Duarte Paulo C; Conrad Patricia A; Wilson W David; Ferraro Gregory L; Packham Andrea E; Bowers-Lepore Jeanne; Carpenter Tim E; Gardner Ian A

Departments of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA.

American journal of veterinary research (United States) Aug 2004, 65 (8) p1047-52, ISSN 0002-9645 Journal Code: 0375011

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

OBJECTIVE: To estimate risk of exposure and age at first exposure to **Sarcocystis neurona** and **Neospora hughesi** and time to maternal antibody decay in foals. ANIMALS: 484 Thoroughbred and Warmblood foals from 4 farms in California. PROCEDURE: Serum was collected before and after

colostrum ingestion and at 3-month intervals thereafter. Samples were tested by use of the indirect fluorescent antibody test; cutoff titers were > or = 40 and > or = 160 for S **neurona** and N **hughesi** , respectively. RESULTS: Risk of exposure to S **neurona** and N **hughesi** during the study were 8.2% and 3.1%, respectively. Annual rate of exposure was 3.1% for S **neurona** and 1.7% for N **hughesi** . There was a significant difference in the risk of exposure to S **neurona** among farms but not in the risk of exposure to N **hughesi** . Median age at first exposure was 1.2 years for S **neurona** and 0.8 years for N **hughesi** . Highest prevalence of antibodies against S **neurona** and N **hughesi** was 6% and 2.1 %, respectively, at a mean age of 1.7 and 1.4 years, respectively. Median time to maternal antibody decay was 96 days for S **neurona** and 91 days for N **hughesi** . There were no clinical cases of equine protozoal myeloenchaphlitis (EPM). CONCLUSIONS AND CLINICAL RELEVANCE: Exposure to S **neurona** and N **hughesi** was low in foals between birth and 2.5 years of age. Maternally acquired antibodies may cause false-positive results for 3 or 4 months after birth, and EPM was a rare clinical disease in horses < or = 2.5 years of age.

Tags: Comparative Study; Research Support, Non-U.S. Gov't

Descriptors: *Antibodies, Protozoan--blood--BL; *Coccidiosis--veterinary --VE; *Horse Diseases--parasitology--PS; * **Neospora** ; * **Sarcocystis** ; Age Factors; Animals; Antibodies, Protozoan--immunology--IM; California; Coccidiosis--immunology--IM; Fluorescent Antibody Technique, Indirect; Horse Diseases--immunology--IM; Horses; Immunity, Maternally-Acquired --immunology--IM; Risk Assessment

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20040831

Record Date Completed: 20041109

3/9/3

15006774 PMID: 14533680

Prevalence of antibodies to Neospora **caninum**, Sarcocystis neurona , **and Toxoplasma gondii in wild horses from central Wyoming.**

Dubey J P; Mitchell S M; Morrow J K; Rhyan J C; Stewart L M; Granstrom D E; Romand S; Thulliez P; Saville W J; Lindsay D S

Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, Agricultural Research Service, United States Department of Agriculture, Building 1001, Beltsville, Maryland 20705-2350, USA. jdubey@anri.barc.usda.gov

Journal of parasitology (United States) Aug 2003, 89 (4) p716-20, ISSN 0022-3395 Journal Code: 7803124

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Sarcocystis neurona , **Neospora** caninum, N. **hughesi** , and Toxoplasma gondii are 4 related coccidians considered to be associated with encephalomyelitis in horses. The source of infection for N. **hughesi** is unknown, whereas opossums, dogs, and cats are the definitive hosts for S. **neurona** , N. caninum, and T. gondii, respectively. Seroprevalence of these coccidians in 276 wild horses from central Wyoming outside the known range of the opossum (Didelphis virginiana) was determined. Antibodies to T. gondii were found only in 1 of 276 horses tested with the modified

agglutination test using 1:25, 1:50, and 1:500 dilutions. Antibodies to N. caninum were found in 86 (31.1%) of the 276 horses tested with the **Neospora** agglutination test--the titers were 1:25 in 38 horses, 1:50 in 15, 1:100 in 9, 1:200 in 8, 1:400 in 4, 1:800 in 2, 1:1,600 in 2, 1:3,200 in 2, and 1:12,800 in 1. Antibodies to S. **neurona** were assessed with the serum immunoblot; of 276 horses tested, 18 had antibodies considered specific for S. **neurona** . Antibodies to S. **neurona** also were assessed with the S. **neurona** direct agglutination test (SAT). Thirty-nine of 265 horses tested had SAT antibodies--in titers of 1:50 in 26 horses and 1:100 in 13. The presence of S. **neurona** antibodies in horses in central Wyoming suggests that either there is cross-reactivity between S. **neurona** and some other infection or a definitive host other than opossum is the source of infection. In a retrospective study, S. **neurona** antibodies were not found by immunoblot in the sera of 243 horses from western Canada outside the range of D. virginiana.

Tags: Female; Male

Descriptors: *Antibodies, Protozoan--blood--BL; *Coccidiosis--veterinary --VE; *Horse Diseases--epidemiology--EP; * **Neospora** --immunology--IM; * **Sarcocystis** --immunology--IM; *Toxoplasma--immunology--IM; Agglutination Tests--veterinary--VE; Animals; Coccidiosis--epidemiology--EP; Horse Diseases--immunology--IM; Horses; Manitoba--epidemiology--EP; Sarcocystosis --epidemiology--EP; Sarcocystosis--veterinary--VE; Saskatchewan--epidemiology--EP; Seroepidemiologic Studies; Toxoplasmosis, Animal--epidemiology--EP ; Wyoming--epidemiology--EP

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20031009

Record Date Completed: 20031023

3/9/4

DIALOG(R)File 155:MEDLINE(R)

14560240 PMID: 12537119

Qualitative evaluation of selective tests for detection of Neospora hughesi **antibodies in serum and cerebrospinal fluid of experimentally infected horses.**

Packham Andrea E; Conrad Patricia A; Wilson W David; Jeanes Lisa V; Sverlow Karen W; Gardner Ian A; Daft Barbara M; Marsh Antoinette E; Blagburn Byron L; Ferraro Gregory L; Barr Bradd C

Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, California 95616, USA. aepackham@ucdavis.edu

Journal of parasitology (United States) Dec 2002, 88 (6) p1239-46, ISSN 0022-3395 Journal Code: 7803124

Publishing Model Print

Document type: Evaluation Studies; Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Neospora hughesi is a newly recognized protozoan pathogen in horses that causes a myeloencephalitis similar to **Sarcocystis neurona** . There are no validated serologic tests using the gold standard sera that are currently available to detect specific N. **hughesi** antibodies and, thus, no tests available to detect antemortem exposure or estimate seroprevalence in the horse. The objectives of the present study were to establish a bank of gold standard equine sera through experimental infections with N.

hughesi and to assess several serologic tests for the detection of related protozoan antibodies. Seven horses were inoculated with N. **hughesi** tachyzoites, and 7 horses received uninfected cell culture material. The horses were monitored, and blood and cerebrospinal fluid were collected repeatedly over a 4-mo period. With the sera, 4 different serologic techniques were evaluated. including a whole-parasite lysate enzyme-linked immunosorbent assay (ELISA), a recombinant protein ELISA, a modified direct agglutination test, and an indirect fluorescent antibody test. Qualitative and quantitative evaluation of the results showed that the N. **hughesi** indirect fluorescent antibody test (IFAT) consistently discriminated between experimentally infected and noninfected horses, using a cutoff of 1:640. Sera from 3 naturally infected horses had titers >1:640. Cerebrospinal fluid in all but I infected horse had very low N. **hughesi** IFAT titers (<1:160), starting at postinoculation day 30.

Tags: Female; Male; Research Support, Non-U.S. Gov't

Descriptors: *Antibodies, Protozoan--blood--BL; *Antibodies, Protozoan --cerebrospinal fluid--CF; *Coccidiosis--veterinary--VE; *Horse Diseases --diagnosis--DI; * **Neospora** --immunology--IM; Agglutination Tests --veterinary--VE; Animals; Coccidiosis--diagnosis--DI; Coccidiosis --immunology--IM; Encephalomyelitis--diagnosis--DI; Encephalomyelitis --immunology--IM; Encephalomyelitis--veterinary--VE; Enzyme-Linked Immunosorbent Assay--methods--MT; Enzyme-Linked Immunosorbent Assay --veterinary--VE; Fluorescent Antibody Technique, Indirect--veterinary--VE; Horse Diseases--immunology--IM; Horses; Random Allocation; Sensitivity and Specificity

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20030122

Record Date Completed: 20030128

3/9/5

DIALOG(R)File 155:MEDLINE(R)

14257575 PMID: 12062508

Seroprevalence of Neospora , **Toxoplasma gondii and** Sarcocystis neurona **antibodies in horses from Jeju island, South Korea.**

Gupta G D; Lakritz J; Kim Jae-Hoon; Kim Dae-Yong; Kim Jin-Kap; Marsh A E

Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Connaway Hall, 1600 East Rollins Dr., Columbia, MO 65211, USA.

Veterinary parasitology (Netherlands) Jun 26 2002, 106 (3) p193-201, ISSN 0304-4017 Journal Code: 7602745

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Parasite-specific antibody responses to **Neospora** spp. and Toxoplasma gondii, antigens were detected using the indirect fluorescent antibody test (IFAT) and immunoblot analysis in a korean equine population located on Jeju island, South Korea (126 degrees 12' E and 33 degrees 34' N). For comparison, a naturally infected **Neospora hughesi** horse and an experimentally inoculated T. gondii equid (pony) were used. In addition, all samples were tested for antibodies to **Sarcocystis neurona** by immunoblot analysis. A total of 191 serum samples from clinically normal horses were evaluated. Only 2% (4 out of 191) and 2.6% (5 out of 191) of

the samples had showed reactivity at 1:100 using the IFAT for **Neospora** spp. and T. gondii, respectively. For T. gondii, two samples matched the antigen banding pattern of the positive control by immunoblot analysis. No sample was positive for N. **hughesi** by immunoblot analysis in this study. Overall, there was a 1% seroprevalence for T. gondii antibodies in the horses tested based on immunoblot analysis. The seroprevalence for S. **neurona** and N. **hughesi** antibodies was 0%. We concluded that these horses are either not routinely exposed to these parasites or antibody titers are not sufficiently elevated to be detectable. It is most likely the former explanation since Jeju island equine farms are isolated from the main land, and the horses were all less than 3 years of age. This naive population of horses could be useful when evaluating S. **neurona** serodiagnostic tests or evaluating potential S. **neurona** vaccines since exposure risks to S. **neurona** and closely related parasites are negligible.

Tags: Female; Male; Research Support, Non-U.S. Gov't

Descriptors: *Coccidiosis--veterinary--VE; *Horse Diseases--parasitology --PS; *Sarcocystidae--immunology--IM; *Sarcocystosis--veterinary--VE; *Toxoplasmosis, Animal--parasitology--PS; Animals; Antibodies, Protozoan --biosynthesis--BI; Antibodies, Protozoan--blood--BL; Blotting, Western --veterinary--VE; Coccidiosis--epidemiology--EP; Coccidiosis--parasitology --PS; Enzyme-Linked Immunosorbent Assay--veterinary--VE; Geography; Horse Diseases--epidemiology--EP; Horses; Korea--epidemiology--EP; **Neospora** --immunology--IM; **Neospora** --isolation and purification--IP; Sarcocystidae--isolation and purification--IP; **Sarcocystis**0 --immunology --IM; **Sarcocystis** --isolation and purification--IP; Sarcocystosis --epidemiology--EP; Sarcocystosis--parasitology--PS; Seroepidemiologic Studies; Toxoplasma--immunology--IM; Toxoplasma --isolation and purification--IP; Toxoplasmosis, Animal--epidemiology--EP

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20020613

Record Date Completed: 20020815

3/9/6

DIALOG(R)File 155:MEDLINE(R)

13609097 PMID: 11223207

Prevalence of Neospora hughesi **and** Sarcocystis neurona **antibodies in horses from various geographical locations.**

Vardeleon D; Marsh A E; Thorne J G; Loch W; Young R; Johnson P J

Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia 65211, USA.

Veterinary parasitology (Netherlands) Feb 26 2001, 95 (2-4) p273-82, ISSN 0304-4017 Journal Code: 7602745

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Parasite-specific antibody responses to **Neospora** antigens were detected using the immunofluorescent antibody test (IFAT) and immunoblot analysis in select equine populations. For comparison, a naturally infected **Neospora** **hughesi** horse and an experimentally inoculated **Neospora** caninum horse were used. In addition, all samples were tested for antibodies to **Sarcocystis** **neurona** by immunoblot analysis. A total of 208 samples was

evaluated. The equine populations were derived from five distinct geographic regions. Locations were selected based on distribution of Didelphis virginiana, the native North American opossum which serves as the definitive host for S. **neurona** . Only 11% of the samples that had positive titers of 1:100 using the IFAT were also positive for antibodies by immunoblot analysis in this study. Overall, there was a 2% seroprevalence for **Neospora** antibodies in all horses tested based on immunoblot analysis described. The seroprevalence for S. **neurona** antibodies varied from 0% (New Zealand and Montana) to 54% (Missouri). We concluded that, in testing for antibodies against **Neospora** antigens using either IFAT or immunoblot analysis, as described, positive results should not be attributed to the presence of antibodies to S. **neurona** .

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Antibodies, Protozoan--analysis--AN; *Horse Diseases --immunology--IM; * **Neospora** --immunology--IM; * **Sarcocystis** --immunology --IM; *Sarcocystosis--veterinary--VE; Animals; Antibodies, Protozoan --biosynthesis--BI; Cross Reactions; Electrophoresis, Polyacrylamide Gel --veterinary--VE; Fluorescent Antibody Technique, Indirect--veterinary--VE; Horse Diseases--epidemiology--EP; Horse Diseases--parasitology--PS; Horses ; Missouri--epidemiology--EP; Montana--epidemiology--EP; New Zealand --epidemiology--EP; Opossums--parasitology--PS; Sarcocystosis--epidemiology --EP; Sarcocystosis--immunology--IM; Seroepidemiologic Studies

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20010306

Record Date Completed: 20010621

3/9/7

DIALOG(R)File 155:MEDLINE(R)

12685232 PMID: 10608440

Prevalence of antibodies to Neospora **sp. in horses from Alabama and characterisation of an isolate recovered from a naturally infected horse [corrected]**

Cheadle M A; Lindsay D S; Rowe S; Dykstra C C; Williams M A; Spencer J A; Toivio-Kinnucan M A; Lenz S D; Newton J C; Rolsma M D; Blagburn B L

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849, USA.

International journal for parasitology (ENGLAND) Oct 1999, 29 (10) p1537-43, ISSN 0020-7519 Journal Code: 0314024

Publishing Model Print; Erratum in Int J Parasitol 2000 Apr 24;30(5) 677

Document type: Case Reports; Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

An IFAT was used to determine the prevalence of **Neospora** -specific IgG antibodies in serum from Alabama horses. Serum samples (n = 536) were from asymptomatic horses routinely submitted for equine infectious anaemia virus infection testing. We also subjected a 13-year-old horse with CNS disease to necropsy examination for isolation and in vitro cultivation of protozoal organisms. In antemortem tests, this horse was positive for antibodies to **Neospora** sp. in the IFAT and western immunoblot. Results of the prevalence survey indicated that IgG antibodies to **Neospora** were present in 62 (11.5%) of the 536 serum samples. Endpoint titres for the positive samples were 1:50 (35/6.5%), 1:100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). Tachyzoites were first seen in cultured bovine turbinate cells 32

days after inoculation with spinal cord homogenates from the horse with CNS disease. Tachyzoites reacted with known N. caninum-positive serum from horses, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or equine anti- **Sarcocystis neurona** serum. Ultrastructural features of tachyzoites and results of comparison of tachyzoite immunodominant proteins revealed that they were identical to those of N. **hughesi** , a species described recently from a naturally infected horse. The isolate recovered from the naturally infected horse in the present study (designated NA1) is thought to be an isolate of N. **hughesi** , although confirmation of this awaits additional molecular characterisation. These results provide some additional evidence that N. **hughesi** is a valid species and that **Neospora** infections in horses may occur in widely separated geographic regions of the United States.
Tags: Female; Research Support, Non-U.S. Gov't
Descriptors: *Antibodies, Protozoan--blood--BL; *Coccidiosis--veterinary --VE; *Horse Diseases--epidemiology--EP; * **Neospora** --immunology--IM; * **Neospora** --isolation and purification--IP; Animals; Antibodies, Protozoan --immunology--IM; Cattle; Coccidiosis--epidemiology--EP; Coccidiosis --parasitology--PS; Dogs; Fluorescent Antibody Technique, Indirect; Horse Diseases--parasitology--PS; Horses; Mice; Myelitis--parasitology--PS; Myelitis--veterinary--VE; **Neospora** --ultrastructure--UL; Prevalence; Spinal Cord--parasitology--PS
CAS Registry No.: 0 (Antibodies, Protozoan)
Record Date Created: 20000120
Record Date Completed: 20000120
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$1.47 7 Type(s) in Format 9
$1.47 7 Types
$4.45 Estimated cost File155
$0.53 TELNET
$4.98 Estimated cost this search
$4.98 Estimated total session cost 1.086 DialUnits

SYSTEM:OS - DIALOG OneSearch
You have 26 files in your file list.
(To see file names, coverage dates, and copyright notices, enter SHOW FILES.)

Set	Items	Description

Added File(s): 5, 34, 35, 48, 65, 71, 73, 91, 94, 98, 135, 144, 149, 156, 159, 162, 164, 172, 266, 369, 370, 399, 434, 444, 467
Previous sets have been retained; enter DISPLAY SETS to view them.
? ds

Set	Items	Description
S1	15	NEOSPORA? AND HUGHESI?
S2	192	SARCOCYSTIS? AND NEURONA?
S3	7	S1 AND S2

? repeat
5062 NEOSPORA?
280 HUGHESI?
S1 111 NEOSPORA? AND HUGHESI?
7608 SARCOCYSTIS?
809310 NEURONA?

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      S2     1221  SARCOCYSTIS? AND NEURONA?
              111  S1
             1221  S2
      S3       53  S1 AND S2
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...completed examining records
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? t s4/6/all
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4/6/1 (Item 1 from file: 155)
17396919 PMID: 15715226
Risk of transplacental transmission of Sarcocystis neurona **and** Neospora hughesi **in California horses.**
Dec 2004

4/6/2 (Item 2 from file: 155)
15462098 PMID: 15334837
Risk of postnatal exposure to Sarcocystis neurona **and** Neospora hughesi **in horses.**
Aug 2004

4/6/3 (Item 3 from file: 155)
15006774 PMID: 14533680
Prevalence of antibodies to Neospora **caninum**, Sarcocystis neurona , **and Toxop**lasma gondii in wild horses from central Wyoming.
Aug 2003

4/6/4 (Item 4 from file: 155)
14560240 PMID: 12537119
Qualitative evaluation of selective tests for detection of Neospora hughesi **antibodies in serum and cerebrospinal fluid of experimentally infected horses.**
Dec 2002

4/6/5 (Item 5 from file: 155)
14257575 PMID: 12062508
Seroprevalence of Neospora , **Toxoplasma gondii and** Sarcocystis neurona **antibodies in horses from Jeju island, South Korea.**
Jun 26 2002

4/6/6 (Item 6 from file: 155)
13609097 PMID: 11223207
Prevalence of Neospora hughesi **and** Sarcocystis neurona **antibodies in horses from various geographical locations.**
Feb 26 2001

4/6/7 (Item 7 from file: 155)
12685232 PMID: 10608440
Prevalence of antibodies to Neospora **sp. in horses from Alabama and characterisation of an isolate recovered from a naturally infected horse**

[corrected]
Oct 1999

4/6/8 (Item 1 from file: 5)
0013946402 BIOSIS NO.: 200200539913
Clinical diagnosis of equine protozoal myeloencephalitis (EPM)
2002

4/6/9 (Item 2 from file: 5)
0012349659 BIOSIS NO.: 200000067972
Prevalence of antibodies to Neospora **Caninum in dogs**
1999

4/6/10 (Item 1 from file: 34)
13487971 Genuine Article#: 886MK Number of References: 24
Title: Sera antibodies to Neospora **caninum in Chilean horses** (ABSTRACT AVAILABLE)
Publication date: 20040000

4/6/11 (Item 2 from file: 34)
13069501 Genuine Article#: 845NY Number of References: 18
Title: Seroprevalence of Neospora **spp. in asymptomatic horses in Italy** (ABSTRACT AVAILABLE)
Publication date: 20040813

4/6/12 (Item 3 from file: 34)
12773523 Genuine Article#: 819YH Number of References: 34
Title: Evaluation and comparison of an indirect fluorescent antibody test for detection of antibodies to Sarcocystis neurona **, using serum and cerebrospinal fluid of naturally and experimentally infected, and vaccinated horses** (ABSTRACT AVAILABLE)
Publication date: 20040400

4/6/13 (Item 4 from file: 34)
10609079 Genuine Article#: 547BR Number of References: 21
Title: Seroprevalence of antibodies against Neospora **caninum in diagnostic equine serum samples and their possible association with fetal loss** (ABSTRACT AVAILABLE)
Publication date: 20020502

4/6/14 (Item 5 from file: 34)
08978843 Genuine Article#: 352HC Number of References: 29
Title: Neospora hughesi **: experimental infections in mice, gerbils, and dogs** (ABSTRACT AVAILABLE)
Publication date: 20000920

4/6/15 (Item 1 from file: 35)
01992302 ORDER NO: AADAA-I1418025
Detection of Sarcocystis neurona 1infection in horses

Year: 2003

4/6/16 (Item 1 from file: 399)
DIALOG(R)File 399:(c) 2005 American Chemical Society. All rts. reserv.

Praziquantel compounds for treating diseases due to sarcocystis, neospora, toxoplasma and isospora

4/6/17 (Item 2 from file: 399)
DIALOG(R)File 399:(c) 2005 American Chemical Society. All rts. reserv.

Decoquinate, 4-hydroxyquinolones and naphthoquinones for the prevention and treatment of equine protozoal myeloencephalitis caused by sarcocystis neurona hughesi and other apicomplexan protozoans

4/6/18 (Item 3 from file: 399)
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Equine protozoal myeloencephalitis vaccine

4/6/19 (Item 4 from file: 399)
DIALOG(R)File 399:(c) 2005 American Chemical Society. All rts. reserv.

Triazinones for treatment of diseases due to Sarcosystis, Neospora and Toxoplasma

4/6/20 (Item 5 from file: 399)
DIALOG(R)File 399:(c) 2005 American Chemical Society. All rts. reserv.

Triazineone compounds for treating diseases due to Sarcosystis, Neospora and Toxoplasma

4/6/21 (Item 6 from file: 399)
DIALOG(R)File 399:(c) 2005 American Chemical Society. All rts. reserv.

Triazineone compounds for treating diseases due to Sarcocystis, Neospora, and Toxoplasma

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    $0.26  TELNET
   $11.82  Estimated cost this search
   $16.80  Estimated total session cost.  2.063 DialUnits

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You are now logged offWelcome to DIALOG
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Dialog level 05.05.00D

Reconnected in file OS 13jun05 14:41:07
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SYSTEM:OS  - DIALOG OneSearch
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         (c) format only 2005 The Dialog Corp.
  File   5:Biosis Previews(R) 1969-2005/Jun W1
         (c) 2005 BIOSIS
  File  34:SciSearch(R) Cited Ref Sci 1990-2005/Jun W1
         (c) 2005 Inst for Sci Info
  File  35:Dissertation Abs Online 1861-2005/May
         (c) 2005 ProQuest Info&Learning
  File  48:SPORTDiscus 1962-2005/Nov
         (c) 2005 Sport Information Resource Centre
  File  65:Inside Conferences 1993-2005/Jun W2
         (c) 2005 BLDSC all rts. reserv.
  File  71:ELSEVIER BIOBASE 1994-2005/Jun W1
         (c) 2005  Elsevier Science B.V.
  File  73:EMBASE 1974-2005/Jun W1
         (c) 2005 Elsevier Science B.V.
  File  91:MANTIS(TM) 1880-2005/May
         2005 (c) Action Potential
  File  94:JICST-EPlus 1985-2005/Apr W4
         (c)2005 Japan Science and Tech Corp(JST)
  File  98:General Sci Abs/Full-Text 1984-2004/Dec
         (c) 2005 The HW Wilson Co.
  File 135:NewsRx Weekly Reports 1995-2005/Jun W1
         (c) 2005 NewsRx
*File 135: New newsletters are now added. See Help News135 for the
complete list of newsletters.
  File 144:Pascal 1973-2005/Jun W1
         (c) 2005 INIST/CNRS
  File 149:TGG Health&Wellness DB(SM) 1976-2005/Jun W1
         (c) 2005 The Gale Group
  File 156:ToxFile 1965-2005/Jun W2
         (c) format only 2005 The Dialog Corporation
*File 156: ToxFile has been reloaded with the 2005 MeSH.
Please see HELP NEWS 156 for details.
  File 159:Cancerlit 1975-2002/Oct
         (c) format only 2002 Dialog Corporation
*File 159: Cancerlit is no longer updating.
Please see HELP NEWS159.
  File 162:Global Health 1983-2005/May
         (c) 2005 CAB International
  File 164:Allied & Complementary Medicine 1984-2005/Jun
         (c) 2005 BLHCIS
  File 172:EMBASE Alert 2005/Jun 08
         (c) 2005 Elsevier Science B.V.
  File 266:FEDRIP 2005/Jun
         Comp & dist by NTIS, Intl Copyright All Rights Res
  File 369:New Scientist 1994-2005/Apr W4
         (c) 2005 Reed Business Information Ltd.
  File 370:Science 1996-1999/Jul W3
         (c) 1999 AAAS
*File 370: This file is closed (no updates). Use File 47 for more current
information.
  File 399:CA SEARCH(R) 1967-2005/UD=14225
         (c) 2005 American Chemical Society
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*File 399: Use is subject to the terms of your user/customer agreement.
Alert feature enhanced for multiple files, etc. See HELP ALERT.
  File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
        (c) 1998 Inst for Sci Info
  File 444:New England Journal of Med. 1985-2005/May W5
        (c) 2005 Mass. Med. Soc.
  File 467:ExtraMED(tm) 2000/Dec
        (c) 2001 Informania Ltd.
*File 467: F467 no longer updates; see Help News467.

      Set Items Description
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Cost is in DialUnits
? ds
Terminal set to DLINK
? t s4/9/7

Set   Items   Description
S1   111   NEOSPORA? AND HUGHESI?
S2   1221   SARCOCYSTIS? AND NEURONA?
S3   53   S1 AND S2
S4   21   RD (unique items)
? t s4/9/7

 4/9/7   (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2005 The Dialog Corp. All rts. reserv.

12685232   PMID: 10608440
   Prevalence of antibodies to Neospora **sp. in horses from Alabama and characterisation of an isolate recovered from a naturally infected horse [corrected]**
  Cheadle M A; Lindsay D S; Rowe S; Dykstra C C; Williams M A; Spencer J A; Toivio-Kinnucan M A; Lenz S D; Newton J C; Rolsma M D; Blagburn B L
  Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849, USA.
  International journal for parasitology (ENGLAND) Oct 1999, 29 (10) p1537-43, ISSN 0020-7519 Journal Code: 0314024
  Publishing Model Print; Erratum in Int J Parasitol 2000 Apr 24;30(5) 677
  Document type: Case Reports; Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: MEDLINE; Completed
  Subfile: INDEX MEDICUS
  An IFAT was used to determine the prevalence of **Neospora** -specific IgG antibodies in serum from Alabama horses. Serum samples (n = 536) were from asymptomatic horses routinely submitted for equine infectious anaemia virus infection testing. We also subjected a 13-year-old horse with CNS disease to necropsy examination for isolation and in vitro cultivation of protozoal organisms. In antemortem tests, this horse was positive for antibodies to **Neospora** sp. in the IFAT and western immunoblot. Results of the prevalence survey indicated that IgG antibodies to **Neospora** were present in 62 (11.5%) of the 536 serum samples. Endpoint titres for the positive samples were 1:50 (35/6.5%), 1:100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). Tachyzoites were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the horse with CNS disease. Tachyzoites reacted with known N. caninum-positive serum from horses, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or equine anti- **Sarcocystis neurona** serum. Ultrastructural

features of tachyzoites and results of comparison of tachyzoite immunodominant proteins revealed that they were identical to those of N. **hughesi** , a species described recently from a naturally infected horse. The isolate recovered from the naturally infected horse in the present study (designated NA1) is thought to be an isolate of N. **hughesi** , although confirmation of this awaits additional molecular characterisation. These results provide some additional evidence that N. **hughesi** is a valid species and that **Neospora** infections in horses may occur in widely separated geographic regions of the United States.
Tags: Female; Research Support, Non-U.S. Gov't
Descriptors: *Antibodies, Protozoan--blood--BL; *Coccidiosis--veterinary --VE; *Horse Diseases--epidemiology--EP; * **Neospora** --immunology--IM; * **Neospora** --isolation and purification--IP; Animals; Antibodies, Protozoan --immunology--IM; Cattle; Coccidiosis--epidemiology--EP; Coccidiosis --parasitology--PS; Dogs; Fluorescent Antibody Technique, Indirect; Horse Diseases--parasitology--PS; Horses; Mice; Myelitis--parasitology--PS; Myelitis--veterinary--VE; **Neospora** --ultrastructure--UL; Prevalence; Spinal Cord--parasitology--PS
CAS Registry No.: 0 (Antibodies, Protozoan)
Record Date Created: 20000120
Record Date Completed: 20000120
? t s4/3/18

4/3/18 (Item 3 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

135343283 CA: 135(24)343283y PATENT
Equine protozoal myeloencephalitis vaccine
INVENTOR(AUTHOR): Bigbie, Rocky Barry; Ng, Terry Kaleung; Whalen, Joseph Wilson, Jr.
LOCATION: USA
ASSIGNEE: American Home Products Corporation
PATENT: PCT International ; WO 200180885 A2 DATE: 20011101
APPLICATION: WO 2001US40527 (20010413) *US PV199435 (20000425) *US PV278695 (20010326)
PAGES: 31 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: A61K-039/00A
DESIGNATED COUNTRIES: AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; BZ; CA; CH; CN; CO; CR; CU; CZ; DE; DK; DM; DZ; EE; ES; FI; GB; GD; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA; MD; MG; MK; MN; MW; MX; MZ; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; TZ; UA; UG; UZ; VN; YU; ZA; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM DESIGNATED REGIONAL: GH; GM; KE; LS; MW; MZ; SD; SL; SZ; TZ; UG ; ZW; AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; TR; BF; BJ; CF; CG; CI; CM; GA; GN; GW; ML; MR; NE; SN; TD; TG
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13jun05 14:41:10 User228206 Session D2455.4
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$0.26 Estimated cost File155
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     $5.77  Estimated total session cost   0.380 DialUnits

File 411:DIALINDEX(R)

DIALINDEX(R)
   (c) 2005 The Dialog Corporation

*** DIALINDEX search results display in an abbreviated ***
*** format unless you enter the SET DETAIL ON command. ***
? sf allscience
   You have 288 files in your file list.
   (To see banners, use SHOW FILES command)
? s neurona? (100n) hughesi (100n) (immunize or vaccin? or inject? or
```

```
merozoite? or tachyzoite?)

Your SELECT statement is:
   s neurona? (100n) hughesi (100n) (immunize or vaccin? or inject? or
merozoite? or tachyzoite?)

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              1    10: AGRICOLA_70-2005/Jun
              3    34: SciSearch(R) Cited Ref Sci_1990-2005/Jun W1
              1    35: Dissertation Abs Online_1861-2005/May
              3    50: CAB Abstracts_1972-2005/May
              3    71: ELSEVIER BIOBASE_1994-2005/Jun W1
              3    73: EMBASE_1974-2005/Jun W1
       Examined  50 files
              1   144: Pascal_1973-2005/Jun W1
              3   155: MEDLINE(R)_1951-2005/Jun W2
              2   162: Global Health_1983-2005/May
              1   185: Zoological Record Online(R)_1978-2005/Jun
       Examined 100 files
              1   292: GEOBASE(TM)_1980-2005/May B1
              2   340: CLAIMS(R)/US Patent_1950-05/Jun 09
              1   342: Derwent Patents Citation Indx_1978-05/200536
              2   349: PCT FULLTEXT_1979-2005/UB=20050609,UT=20050602
       Examined 150 files
              1   357: Derwent Biotech Res.__1982-2005/Jun W1
              4   440: Current Contents Search(R)_1990-2005/Jun 10
       Examined 200 files
              8   654: US Pat.Full._1976-2005/Jun 09
       Examined 250 files

   18 files have one or more items; file list includes 288 files.

?
? save temp
Temp SearchSave "TG66909924" stored
? rf
Your last SELECT statement was:
   S NEURONA? (100N) HUGHESI (100N) (IMMUNIZE OR VACCIN? OR INJECT? OR MER-
OZOITE? OR TACHYZOITE?)
Ref        Items   File
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N2             4   440: Current Contents Search(R)_1990-2005/Jun 10
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N4             3    34: SciSearch(R) Cited Ref Sci_1990-2005/Jun W1
N5             3    50: CAB Abstracts_1972-2005/May
N6             3    71: ELSEVIER BIOBASE_1994-2005/Jun W1
N7             3    73: EMBASE_1974-2005/Jun W1
N8             3   155: MEDLINE(R)_1951-2005/Jun W2
N9             2   162: Global Health_1983-2005/May
N10            2   340: CLAIMS(R)/US Patent_1950-05/Jun 09
   18 files have one or more items; file list includes 288 files.

        - Enter P or PAGE for more -
? p
Your last SELECT statement was:
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OZOITE? OR TACHYZOITE?)
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N15            1   185: Zoological Record Online(R)_1978-2005/Jun
N16            1   292: GEOBASE(TM)_1980-2005/May B1

N17            1   342: Derwent Patents Citation Indx_1978-05/200536
N18            1   357: Derwent Biotech Res.__1982-2005/Jun W1
N19            0     2: INSPEC_1969-2005/Jun W1
N20            0     6: NTIS_1964-2005/Jun W1
   18 files have one or more items; file list includes 288 files.

        - Enter P or PAGE for more -
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Your last SELECT statement was:
   S NEURONA? (100N) HUGHESI (100N) (IMMUNIZE OR VACCIN? OR INJECT? OR MER-
OZOITE? OR TACHYZOITE?)
Ref        Items   File
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N21            0     8: Ei Compendex(R)_1970-2005/Jun W1
N22            0     9: Business & Industry(R)_Jul/1994-2005/Jun 13
N23            0    15: ABI/Inform(R)_1971-2005/Jun 13
N24            0    16: Gale Group PROMT(R)_1990-2005/Jun 13
N25            0    18: Gale Group F&S Index(R)_1988-2005/Jun 13
N26            0    19: Chem.Industry Notes_1974-2005/ISS 200523
N27            0    20: Dialog Global Reporter_1997-2005/Jun 13
N28            0    25: Weldasearch-19662005/May
N29            0    29: Meteorological and GeoAstrophysical Abstracts_2005
N30            0    31: World Surface Coatings Abs_1976-2005/May
   18 files have one or more items; file list includes 288 files.

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OZOITE? OR TACHYZOITE?)
Ref        Items   File
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N31            0    36: MetalBase_1965-20050613
N32            0    40: Enviroline(R)_1975-2005/May
N33            0    42: Pharmaceuticl News Idx_1974-2005/May W5
N34            0    47: Gale Group Magazine DB(TM)_1959-2005/Jun 13
N35            0    48: SPORTDiscus_1962-2005/Nov
N36            0    49: PAIS Int._1976-2005/Feb
N37            0    51: Food Sci.&Tech.Abs_1969-2005/Jun W2
N38            0    52: TSCA Chemical Substances Inventory_2003/OCT,
N39            0    53: FOODLINE(R): Science Sight_1972-2005/Jun 13
N40            0    54: FOODLINE(R): Market Sight_1979-2005/Jun 13
   18 files have one or more items; file list includes 288 files.

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    $12.26  Estimated cost this search
    $18.03  Estimated total session cost   4.705 DialUnits

SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R) 1951-2005/Jun W2
         (c) format only 2005 The Dialog Corp.
  File 654:US Pat.Full. 1976-2005/Jun 09
         (c) Format only 2005 The Dialog Corp.
  File   5:Biosis Previews(R) 1969-2005/Jun W1
         (c) 2005 BIOSIS
  File  50:CAB Abstracts 1972-2005/May
         (c) 2005 CAB International
  File  73:EMBASE 1974-2005/Jun W1
         (c) 2005 Elsevier Science B.V.
  File 340:CLAIMS(R)/US Patent 1950-05/Jun 09
         (c) 2005 IFI/CLAIMS(R)
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  File  10:AGRICOLA 70-2005/Jun
         (c) format only 2005 The Dialog Corporation
  File  35:Dissertation Abs Online 1861-2005/May
         (c) 2005 ProQuest Info&Learning
  File 144:Pascal 1973-2005/Jun W1
         (c) 2005 INIST/CNRS
  File 342:Derwent Patents Citation Indx 1978-05/200536
         (c) 2005 Thomson Derwent
  File 357:Derwent Biotech Res. _1982-2005/Jun W1
         (c) 2005 Thomson Derwent & ISI

      Set  Items  Description
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Executing TG66909924
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          566699  NEURONA?
             155  HUGHESI
           26005  IMMUNIZE
          664789  VACCIN?
         2666076  INJECT?
           14143  MEROZOITE?
            6266  TACHYZOITE?
      S1      29  NEURONA? (100N) HUGHESI (100N) (IMMUNIZE OR VACCIN? OR
                  INJECT? OR MEROZOITE? OR TACHYZOITE?)
? t s1/3,kwic/all

 1/3,KWIC/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2005 The Dialog Corp. All rts. reserv.

14560240   PMID: 12537119
  Qualitative evaluation of selective tests for detection of Neospora
hughesi antibodies in serum and cerebrospinal fluid of experimentally
infected horses.
  Packham Andrea E; Conrad Patricia A; Wilson W David; Jeanes Lisa V;
Sverlow Karen W; Gardner Ian A; Daft Barbara M; Marsh Antoinette E;
Blagburn Byron L; Ferraro Gregory L; Barr Bradd C
  Department of Pathology, Microbiology and Immunology, School of
Veterinary Medicine, University of California, One Shields Avenue, Davis,
California 95616, USA. aepackham@ucdavis.edu
```

Journal of parasitology (United States) Dec 2002, 88 (6) p1239-46, ISSN 0022-3395 Journal Code: 7803124
Publishing Model Print
Document type: Evaluation Studies; Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed

Neospora **hughesi** is a newly recognized protozoan pathogen in horses that causes a myeloencephalitis similar to Sarcocystis **neurona** . There are no validated serologic tests using the gold standard sera that are currently available to detect specific N. **hughesi** antibodies and, thus, no tests available to detect antemortem exposure or estimate seroprevalence in the...

... were to establish a bank of gold standard equine sera through experimental infections with N. **hughesi** and to assess several serologic tests for the detection of related protozoan antibodies. Seven horses were inoculated with N. **hughesi** **tachyzoites** , and 7 horses received uninfected cell culture material. The horses were monitored, and blood and ...

... indirect fluorescent antibody test. Qualitative and quantitative evaluation of the results showed that the N. **hughesi** indirect fluorescent antibody test (IFAT) consistently discriminated between experimentally infected and noninfected horses, using a...

... titers >1:640. Cerebrospinal fluid in all but I infected horse had very low N. **hughesi** IFAT titers (<1:160), starting at postinoculation day 30.

1/3,KWIC/2 (Item 2 from file: 155)

14257575 PMID: 12062508

Seroprevalence of Neospora, Toxoplasma gondii and Sarcocystis neurona antibodies in horses from Jeju island, South Korea.

Gupta G D; Lakritz J; Kim Jae-Hoon; Kim Dae-Yong; Kim Jin-Kap; Marsh A E
Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Connaway Hall, 1600 East Rollins Dr., Columbia, MO 65211, USA.

Veterinary parasitology (Netherlands) Jun 26 2002, 106 (3) p193-201, ISSN 0304-4017 Journal Code: 7602745
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed

... 126 degrees 12' E and 33 degrees 34' N). For comparison, a naturally infected Neospora **hughesi** horse and an experimentally inoculated T. gondii equid (pony) were used. In addition, all samples were tested for antibodies to Sarcocystis **neurona** by immunoblot analysis. A total of 191 serum samples from clinically normal horses were evaluated...
... banding pattern of the positive control by immunoblot analysis. No sample was positive for N. **hughesi** by immunoblot analysis in this study. Overall, there was a 1% seroprevalence for T. gondii antibodies in the

US
Examiner: Smith, L. F.
Assistant Examiner: Baskar, Padma
Legal Representative: Fulbright & Jaworski, LLP

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6891024	B2	20050510	US 2002140754	20020507
Related Publ	US 20020187517	A1	20021212		
Provisional				US 60-293603	20010524
Provisional				US 60-297810	20010612

US Term Extension: 69 days
Fulltext Word Count: 12075

Summary of the Invention:

...scatter versus log side scatter. Subclones were then tested by an ELISA using S. neurona **merozoite** antigen lysate and host monolayer control lysate coated plates. These clones were expanded, repeated tested ...

...were tested by western blot technique, immunofluorescence antibody assay, and an immunohistochemistry assay on S. **neurona** -infected mouse tissues0085] Immunoblot and immunofluorescence analysis. Culture-derived S. **neurona** , Sarcocystis falcatula, N. **hughesi** or Toxoplasma gondii were lysed in non-reducing sample buffer. The parasite isolates have been ...not lost after periodate treatment. The polyclonal anti-S. neurona antibody reacted with reduced S. **neurona** antigen whereas mAb 2A7-18 and mAb 2G5-2 did not. MAb 2A7-18 reacted with S. **neurona** isolates obtained from horses in California but did not react with one (SN-MU1) of the two S. **neurona** isolates obtained from horses in Missouri; however, mAb 2G5-2 recognized all the S. **neurona** isolates tested. The immunoblots indicate slight differences in the molecular size of this approximately 14...

...were of similar molecular size. When the immunoblots were reprobed with rabbit polyclonal anti-S. **neurona** antibodies, both immunodominant proteins were visible in all the Sarcocystis isolates except SN-MU1. The ...

...were present in concentrations comparable to those of the other Sarcocystis antigens. Toxoplasma gondii, N. **hughesi** or host cell antigens showed no reactivity by immunoblot analysis when probed with mAb 2A7...

...0090] By IFA, mAb 2A7-18 reacted to the surface of live and fixed S. **neurona** **merozoites** but not to the other parasites tested. MAb 2G5-2 required the S. **neurona** **merozoites** to be fixed for reactivity as live staining of parasites showed little reactivity with this...

...In some sections, mAb 2G5-2 labeling was concentrated within intracellular vesicles of S. neurona **merozoites** . When both primary antibodies were diluted 200-fold, the reactivity of mAb 2A7-18 was...

1/3,KWIC/5 (Item 2 from file: 654)
DIALOG(R)File 654:US Pat.Full.

5993734
Derwent Accession: 2005-161937
UTILITY
Detection of sarcocystis neurona
Inventor: Dame, John B., Gainesville, FL, US
Ellison, Siobhan P., Fairfield, FL, US
Yowell, Charles A., Gainesville, FL, US
Assignee: Unassigned
Correspondence Address: SALIWANCHIK LLOYD & SALIWANCHIK;A PROFESSIONAL ASSOCIATION, PO BOX 142950, GAINESVILLE, FL, 32614-2950, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20050037443	A1	20050217	US 2004916046	20040810
Division	US 6808714			US 2001962993	20010924
Provisional				US 60-234676	20000922

Fulltext Word Count: 19644
Description of the Invention:

...responded with an ELISA titer of greater than 1:8,000 to S. neurona cultured **merozoites** . Antibodies to whole mouse immunoglobulin molecule serum levels showed a good response to S. neurona...

...an anti-SnSAG-1 monoclonal antibody was produced by immunizing a mouse with whole S. **neurona** parasites followed by routine fusion with myeloma cells. A well (...was tested and found to contain a monoclonal antibody (isotype IgG[sub]1) to S. **neurona** . The cells were subjected to cloning by limiting dilution and transferred to a 24 well...

...1631 reacted by ELISA at greater than 1:16,000 and immunoblotted whole cell S. **neurona** antigens to a single band but not to host cells. Analysis of the supernatants from...

...contained a monoclonal antibody that specifically bound (a) a 29 kDa band of blotted S. **neurona** antigens, and (b) the surface of whole formalin-fixed or methanol-fixed parasites by IFA...

...1631 antibody was used in an ELISA to determine cross-reactivity with two non-S. **neurona** Apicomplexan parasites known to infect horses. The data indicated that 1631 does not bind Neospora **hughesi** **merozoites** , Toxoplasma gondii **tachyzoites** , or host cells...

...0110] The 1631 antibody preparation reacted with live cells or formalin fixed S. **neurona** but not host cells. Similar results were obtained with methanol fixed parasites. The phase contrast...

...this experiment, pre-immune mouse serum and isotype control, also showed no binding to S. **neurona** by immunofluorescence assay. Post-embedding immunogold labeling of S. **neurona** cultured **merozoites** using 1631 antibodies also showed that the surface of the parasite was labeled. Controls included...

...monoclonal isotype control. The cloned hybridoma 1631 was used to label the surface of S. **neurona** cultured **merozoites** by post-embedding immunogold labeling...

1/3,KWIC/6 (Item 3 from file: 654)
DIALOG(R)File 654:US Pat.Full.

5845932
Derwent Accession: 2002-712484
Utility
Detection of sarcocystis neurona
Inventor: Dame, John B., Gainesville, FL
Ellison, Siobhan P., Fairfield, FL
Yowell, Charles A., Gainesville, FL
Assignee: University of Florida(02), Gainesville, FL
Examiner: Smith, Lynette R. F. (Art Unit: 165)
Assistant Examiner: Baskar, Padma
Law Firm: Saliwanchik, Lloyd & Saliwanchik

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6808714	A	20041026	US 2001962993	20010924

Fulltext Word Count: 19327

Description of the Invention:
...responded with an ELISA titer of greater than 1:8,000 to S. neurona cultured **merozoites** . Antibodies to whole mouse immunoglobulin molecule serum levels showed a good response to S. neurona...

...an anti-SnSAG-1 monoclonal antibody was produced by immunizing a mouse with whole S. **neurona** parasites followed by routine fusion with myeloma cells. A well (1631) from the 96 well...

...was tested and found to contain a monoclonal antibody (isotype IgG[sub]1) to S. **neurona** . The cells were subjected to cloning by limiting dilution and transferred to a 24 well cell S. **neurona** antigens to a single band but not to host cells. Analysis of the supernatants from ...

...contained a monoclonal antibody that specifically bound (a) a 29 kDa band of blotted S. **neurona** antigens, and (b) the surface of whole formalin-fixed or methanol-fixed parasites by IFA...

...1631 antibody was used in an ELISA to determine cross-reactivity with two non-S. **neurona** Apicomplexan parasites known to infect horses. The data indicated that 1631 does not bind Neospora **hughesi** **merozoites** , Toxoplasma gondii **tachyzoites** , or host cells...
...The 1631 antibody preparation reacted with live cells or formalin fixed S. **neurona** but not host cells. Similar results were obtained with methanol fixed parasites. The phase contrast...

...this experiment, pre-immune mouse serum and isotype control, also showed no binding to S. **neurona** by immunofluorescence assay. Post-embedding immunogold labeling of S. **neurona** cultured **merozoites** using 1631 antibodies also showed that the surface of the parasite was labeled. Controls included...

...monoclonal isotype control. The cloned hybridoma 1631 was used to label the surface of S. **neurona** cultured **merozoites** by post-embedding immunogold labeling

1/3,KWIC/7 (Item 4 from file: 654)
DIALOG(R)File 654:US Pat.Full.

5767158 **IMAGE Available
Derwent Accession: 2004-200933
Utility
Animal model for infection by an apicomplexan parasite
Inventor: Ellison, Siobhan P., P.O. Box 970, Fairfield, FL, 32634
Assignee: Unassigned
Examiner: Navarro, Mark (Art Unit: 165)
Law Firm: Allen, Dyer, Doppelt, Milbrath & Gilchrist, P.A.

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6780415	A	20040824	US 2002152960	20020522

Fulltext Word Count: 12713

Description of the Invention:
...can be activated so as to cross the blood brain barrier, can be infected by **merozoites** of Apicomplexan parasites. After merozite infection of the mammalian cells, the infected cells can be...
...serve as models for development of efficacious drug treatments, prophylactic modalities such as drugs and **vaccines** , and diagnostic tests for determination of Apicomplexan infections. Apicomplexan diseases described by the present invention...

...which the models are useful include but are not limited to EPM caused by S. **neurona** , S. dasypus, S. falcatula or N. **hughesi** (hereinafter designated as EPM-producing parasites) or Neosporosis caused by N. **hughesi** , N. caninum, bovine,

Non-exemplary or Dependent Claim(s):
...1, wherein the infected susceptible homologous cell consists of a leukocyte comprising at least a **merozoite** stage of a parasite selected from Sarcocystis **neurona** , Sarcocystis falcatula, and Neurosporosis **hughesi** , said **merozoite** having an unextruded conoid ...

1/3,KWIC/8 (Item 5 from file: 654)
DIALOG(R)File 654:US Pat.Full.

0005447919 **IMAGE Available
Derwent Accession: 2004-200933
Animal model for infection by an apicomplexan parasite
Inventor: Ellison, Siobhan, INV

Correspondence Address: ALLEN, DYER, DOPPELT, MILBRATH, & GILCHRIST, P.A., HENRY ESTEVEZ, 255 S. ORANGE AVE SUITE 1401 P.O.BOX 3791, ORLANDO,, FL, 32802, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20030219381	A1	20031127	US 2002152960	20020522

Fulltext Word Count: 15351

Description of the Invention:
...can be activated so as to cross the blood brain barrier, can be infected by **merozoites** of Apicomplexan parasites. After merozite infection of the mammalian cells, the infected cells can be...
...serve as models for development of efficacious drug treatments, prophylactic modalities such as drugs and **vaccines** , and diagnostic tests for determination of Apicomplexan infections. Apicomplexan diseases described by the present invention...which the models are useful include but are not limited to EPM caused by S. **neurona** , S. dasypus, S. falcatula or N. **hughesi** (hereinafter designated as EPM-producing parasites) or Neosporosis caused by N. **hughesi** , N. caninum, bovine, equine or canine isolates (hereinafter designated as Neospora-producing parasites), Toxoplasmosis caused...

Non-exemplary or Dependent Claim(s):
...or 12 wherein the EPM-producing parasite is selected from the group consisting of S. **neurona** , S. dasypus (sp. S. **neurona**), S. falcatula and N. **hughesi** .
...claim 17 wherein the treatment is selected from the group consisting of a drug, a **vac**cine and a diagnostic...

1/3,KWIC/9 (Item 6 from file: 654)
DIALOG(R)File 654:US Pat.Full.

0005135967
Derwent Accession: 2003-341035
Monoclonal antibodies to Sarcocystis neurona and uses therefor
Inventor: Antoinette Marsh, INV
Correspondence Address: FULBRIGHT & JAWORSKI L.L.P., SUITE 2400 600 CONGRESS AVENUE, AUSTIN, TX, 78701-3271, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20020187517	A1	20021212	US 2002140754	20020507
Provisional				US 60-293603	20010524
Provisional				US 60-297810	20010612

Fulltext Word Count: 14262

Description of the Invention:
...scatter versus log side scatter. Subclones were then tested by an ELISA using S. neurona **merozoite** antigen lysate and host monolayer

control lysate coated plates. These clones were expanded, repeated tested immunohistochemistry assay on S. **neurona** -infected mouse tissues...

...0094] Immunoblot and immunofluorescence analysis. Culture-derived S. **neurona** , Sarcocystis falcatula, N. **hughesi** or Toxoplasma gondii were lysed in non-reducing sample buffer. The parasite isolates have been... not lost after periodate treatment. The polyclonal anti-S. neurona antibody reacted with reduced S. **neurona** antigen whereas mAb 2A7-18 and mAb 2G5-2 did not. MAb 2A7-18 reacted with S. **neurona** isolates obtained from horses in California but did not react with one (SN-MU1) of the two S. **neurona** isolates obtained from horses in Missouri; however, mAb 2G5-2 recognized all the S. **neurona** isolates tested. The immunoblots indicate slight differences in the molecular size of this approximately 14...

...were of similar molecular size. When the immunoblots were reprobed with rabbit polyclonal anti-S. **neurona** antibodies, both immunodominant proteins were visible in all the Sarcocystis isolates except SN-MU1. The ...

...were present in concentrations comparable to those of the other Sarcocystis antigens. Toxoplasma gondii, N. **hughesi** or host cell antigens showed no reactivity by immunoblot analysis when probed with mAb 2A7...0099] By IFA, mAb 2A7-18 reacted to the surface of live and fixed S. **neurona** **merozoites** but not to the other parasites tested. MAb 2G5-2 required the S. **neurona** **merozoites** to be fixed for reactivity as live staining of parasites showed little reactivity with this...

...In some sections, mAb 2G5-2 labeling was concentrated within intracellular vesicles of S. neurona **merozoites** . When both primary antibodies were diluted 200-fold, the reactivity of mAb 2A7-18 was...

1/3,KWIC/10 (Item 7 from file: 654)
DIALOG(R)File 654:US Pat.Full.

0005061084
Derwent Accession: 2002-712484
Detection of sarcocystis neurona
Inventor: John Dame, INV
Siobhan Ellison, INV
Charles Yowell, INV
Correspondence Address: Stanley A. Kim Akerman, Senterfitt & Eidson, P.A., 222 Lakeview Avenue, Suite 400 P.O. Box 3188, West Palm Beach, FL, 33402-3188, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20020115828	A1	20020822	US 2001962993	20010924
Provisional				US 60-234676	20000922

Fulltext Word Count: 23294

Description of the Invention:
...responded with an ELISA titer of greater than 1:8,000 to S. neurona

cultured **merozoites** . Antibodies to whole mouse immunoglobulin molecule serum levels showed a good response to S. neurona...an anti-SnSAG-1 monoclonal antibody was produced by immunizing a mouse with whole S. **neurona** parasites followed by routine fusion with myeloma cells. A well (1631) from the 96 well...

...was tested and found to contain a monoclonal antibody (isotype IgG[sub]1) to S. **neurona** . The cells were subjected to cloning by limiting dilution and transferred to a 24 well...

...1631 reacted by ELISA at greater than 1:16,000 and immunoblotted whole cell S. **neurona** antigens to a single band but not to host cells. Analysis of the supernatants from...

...contained a monoclonal antibody that specifically bound (a) a 29 kDa band of blotted S. **neurona** antigens, and (b) the surface of whole formalin-fixed or methanol-fixed parasites by IFA...

...1631 antibody was used in an ELISA to determine cross-reactivity with two non-S. **neurona** Apicomplexan parasites known to infect horses. The data indicated that 1631 does not bind Neospora **hughesi** **merozoites** , Toxoplasma gondii **tachyzoites** , or host cells0110] The 1631 antibody preparation reacted with live cells or formalin fixed S. **neurona** but not host cells. Similar results were obtained with methanol fixed parasites. The phase contrast...

...this experiment, pre-immune mouse serum and isotype control, also showed no binding to S. **neurona** by immunofluorescence assay. Post-embedding immunogold labeling of S. **neurona** cultured **merozoites** using 1631 antibodies also showed that the surface of the parasite was labeled. Controls included...

...monoclonal isotype control. The cloned hybridoma 1631 was used to label the surface of S. **neurona** cultured **merozoites** by post-embedding immunogold labeling...

1/3,KWIC/11 (Item 8 from file: 654)
DIALOG(R)File 654:US Pat.Full.

0004987264
Derwent Accession: 2002-049244
Equine protozoal myeloencephalitis vaccine
Inventor: Rocky Bigbie, INV
Terry Ng, INV
Joseph Whalen, INV
Assignee: American Home Products Corporation(02), Madison, NJ
Correspondence Address: AMERICAN HOME PRODUCTS CORPORATION, FIVE GIRALDA FARMS PATENT LAW, MADISON, NJ, 07940, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20020041886	A1	20020411	US 2001840485	20010423
Provisional				US 60-199435	20000425
Provisional				US 60-278695	20010326

Fulltext Word Count: 6180

Abstract:
The present invention provides an immunogenically active component comprising inactivated Sarcocystis **neurona** cells and/or inactivated Neospora **hughesi** cells; antigens derived therefrom; DNA derived therefrom; or a mixture; or in combination with other **vaccine** components thereof...

...Further provided are **vaccine** compositions useful for preventing or ameliorating equine protozoal myeloencephalitis infection and disease and a method...

Summary of the Invention:
...0003] Initially EPM was thought to only be caused by Sarcocystis **neurona** . The opossum (Didelphis virginiana) has been identified as the definitive host for this agents. The...

...unknown. The horse ingests feed which has been contaminated with opossum fecal material containing Sarcocystis **neurona** sporocysts. These sporocysts then excyst in the intestinal epithelium of the intermediate and incidental hosts...

...tissues of the host forming sarcocysts. In the case of the aberrant host, the Sarcocystis **neurona** multiply in the Central Nervous System (spinal cord) and fail to encyst. In horses, the only observed forms of Sarcocystis **neurona** have been the meront or **merozoite** .

[...

...0004] Recently Neospora **hughesi** has been identified as a second organism which will cause the EPM clinical disease. Neospora **hughesi** will not only infect the spinal cord as Sarcocystis **neurona** does, but will also colonize the brain. At this point in time the definitive and intermediate hosts for Neospora **hughesi** remain unknown. It is believed that fecal contamination of horse feed or water with sporulated oocysts is the route of horse infection. The oocysts will release **tachyzoites** which will infect cells as do the **merozoites** of Sarcocystis **neurona** .

[...

...0006] There is currently no **vaccine** or approved animal drug product available for the effective treatment of EPM. The currently available... 0010] The present invention provides an immunogenically active component which comprises inactivated Sarcocystis **neurona** cells or inactivated Neospora hughesi ...0016]Sarcocystis neurona or Neospora **hughesi** protozoa are the causative agents of equine protozoal myeloencephalitis (EPM) disease, which is a serious...

...host and apparently become infected when ingesting feed which has been contaminated with the Sarcocystis **neurona** or Neospora **hughesi** protozoans via opossum fecal contamination. EPM disease when untreated will progress from initial numbness of...

...Surprisingly, it has now been found that an immunogenically active component which comprises inactivated Sarcocystis **neurona** cells or antigens, subunit proteins or plasmid DNA; inactivated Neospora **hughesi**

cells or antigens, subunit proteins or plasmid DNA; or mixtures thereof may be administered in...

...composition to prevent or ameliorate EPM disease in equines, particularly horses. Antigens derived from Sarcocystis **neurona** or Neospora **hughesi** may be obtained using conventional procedures such as outer membrane extraction. Plasmid DNA derived from Sarcocystis **neurona** or Neospora **hughesi** may be obtained via isolation from sources such as the fluids or tissues of equine...

...A useful starting isolate for the vaccines of the invention include, for example, for Sarcocystis **neurona** , the isolate designated SN3; other such isolates are those known as SN1, SN2, SN4, SN5...

...Oregon State University, the University of Missouri and others. A culture of one such Sarcocystis **neurona** isolate designated SNg, originally isolated from the intestinal scrapings of the opossum and confirmed to be a representative Sarcocystis **neurona** by ...A useful starting isolate for the vaccines of the invention include, for example, for Neospora **hughesi** , the isolate designated NEQ1; another such isolate is that known as NE1, which has been...
...al, Journal of Parasitology, 84 (5), 1998, pp983-991. A culture of one such Neospora **hughesi** isolate has been deposited with the ATCC and given ATCC Accession No. 209622 (NE1) as...

...decanting the growth media; refeeding the cells with fresh growth media; inoculating the cells with **merozoites** or **tachyzoites** ; after 4-12 days, decanting the growth media; and refeeding the inoculated cells a second...

Exemplary or Independent Claim(s):

1. An immunogenically active component which comprises a member selected from the group consisting of **merozoite** antibody inducing, inactivated Sarcocystis neurona cells; tachyzoite antibody inducing, inactivated Neospora **hughesi** cells; a merozoite or tachyzoite antibody inducing antigen derived from said cells; DNA derived from ...

...first immunogenically active component selected from the group consisting of merozoite antibody inducing, inactivated Sarcocystis **neurona** cells; a merozoite antibody inducing antigen derived from said cells; DNA derived from said cells...

...second immunogenically active component selected from the group consisting of tachyzoite antibody inducing, inactivated Neospora **hughesi** cells; a tachyzoite antibody inducing antigen derived from said cells; DNA derived from said cells from the group consisting of merozoite antibody inducing, inactivated Sarcocystis **neurona** cells; tachyzoite antibody inducing, inactivated Neospora **hughesi** cells; a merozoite or tachyzoite antibody inducing antigen derived from said cells; DNA derived from...
...which comprises a member selected from the group consisting of merezoite antibody inducing, inactivated Sarcocystis **neurona** cells; tachyzoite antibody inducing, inactivated Neospora **hughesi** cells; a merezoite or tachyzoite antibody inducing antigen derived from said cells; DNA derived from...

...first immunogenically active component selected from the group

consisting of merozoite antibody inducing, inactivated Sarcocystis **neurona** cells; a merozoite antibody inducing antigen derived from said cells; DNA derived ...or a mixture thereof; a second immunogenically active component selected from the group consisting of **tachyzoite** antibody inducing, inactivated Neospora **hughesi** cells; a **tachyzoite** antibody inducing antigen derived from said cells; DNA derived from said cells capable of inducing a **tachyzoite** antibody immune response; or a mixture thereof; a pharmacologically acceptable carrier; and optionally an immunogenically...

...23. A method for the cell culture propagation of Sarcocystis **neurona** or Neospora **hughesi** protozoan parasite which comprises: a) growing a monolayer of cells having a confluency of 80%-100%; b) refeeding said cells with supplemented growth media; c) inoculating said cells with **merozoites** or **tachyzoites** ; d) holding the inoculated cells for 4-12 days; e) decanting the supplemented growth media...

Non-exemplary or Dependent Claim(s):

...13. The **vaccine** composition according to claim 12 wherein said adjuvant is a metabolizable oil14. The **vaccine** composition according to claim 13 wherein the pharmacologically acceptable carrier is a balanced salt solution...

...16. The **vaccine** composition according to claim 15 wherein said first immunologically active component comprises inactivated Sarcocystis **neurona** cells and said second immunologically effective component comprises inactivated Neospora **hughesi** cells...

...17. The **vaccine** composition according to claim 15 wherein said first immunologically active component is present in an...

...wherein said second immunologically active component is present in an amount sufficient to produce a **tachyzoite** inducing serum neutralizing antibody response which is protozocidal...

...21. The method according to claim 18 wherein said **vaccine** is administered parenterally

1/3,KWIC/12 (Item 1 from file: 5)

0014153595 BIOSIS NO.: 200300112314

Qualitative evaluation of selective tests for detection of Neospora hughesi antibodies in serum and cerebrospinal fluid of experimentally infected horses.

AUTHOR: Packham Andrea E (Reprint); Conrad Patricia A (Reprint); Wilson W David; Jeanes Lisa V; Sverlow Karen W; Gardner Ian A; Daft Barbara M; Marsh Antoinette E; Blagburn Byron L; Ferraro Gregory L; Barr Bradd C
AUTHOR ADDRESS: Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA, 95616, USA**USA
AUTHOR E-MAIL ADDRESS: aepackham@ucdavis.edu

JOURNAL: Journal of Parasitology 88 (6): p1239-1246 December 2002 2002
MEDIUM: print
ISSN: 0022-3395
DOCUMENT TYPE: Article

RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Neospora **hughesi** is a newly recognized protozoan pathogen in horses that causes a myeloencephalitis similar to Sarcocystis **neurona** . There are no validated serologic tests using the gold standard sera that are currently available to detect specific N. **hughesi** antibodies and, thus, no tests available to detect antemortem exposure or estimate seroprevalence in the...

...were to establish a bank of gold standard equine sera through experimental infections with N. **hughesi** and to assess several serologic tests for the detection of related protozoan antibodies. Seven horses were inoculated with N. **hughesi** **tachyzoites** , and 7 horses received uninfected cell culture material. The horses were monitored, and blood and...

...indirect fluorescent antibody test. Qualitative and quantitative evaluation of the results showed that the N. **hughesi** indirect fluorescent antibody test (IFAT) consistently discriminated between experimentally infected and noninfected horses, using a...

...titers >1:640. Cerebrospinal fluid in all but 1 infected horse had very low N. **hughesi** IFAT titers (<1:160), starting at postinoculation day 30.

1/3,KWIC/13 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0013812616 BIOSIS NO.: 200200406127
Seroprevalence of Neospora, Toxoplasma gondii and Sarcocystis neurona antibodies in horses from Jeju island, South Korea
AUTHOR: Gupta G D; Lakritz J; Kim Jae-Hoon; Kim Dae-Yong; Kim Jin-Kap; Marsh A E (Reprint)
AUTHOR ADDRESS: Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, 1600 East Rollins Dr., Connaway Hall, Columbia, MO, 65211, USA**USA
JOURNAL: Veterinary Parasitology 106 (3): p193-201 26 June, 2002 2002
MEDIUM: print
ISSN: 0304-4017
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

...ABSTRACT: Jeju island, South Korea (126degree12' E and 33degree34' N). For comparison, a naturally infected Neospora **hughesi** horse and an experimentally inoculated T. gondii equid (pony) were used. In addition, all samples were tested for antibodies to Sarcocystis **neurona** by immunoblot analysis. A total of 191 serum samples from clinically normal horses were evaluated...
...banding pattern of the positive control by immunoblot analysis. No sample was positive for N. **hughesi** by immunoblot analysis in this study. Overall, there was a 1% seroprevalence for T. gondii antibodies in the horses tested based on immunoblot analysis. The seroprevalence for S. **neurona** and N. **hughesi** antibodies was 0%. We concluded that these horses are either not routinely exposed to these...

...3 years of age. This naive population of horses could be useful when evaluating S. **neurona** serodiagnostic tests or evaluating potential S. **neurona** **vaccines** since exposure risks to S. **neurona** and closely related parasites are negligible.

1/3,KWIC/14 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0012349659 BIOSIS NO.: 200000067972
Prevalence of antibodies to Neospora Caninum in dogs
AUTHOR: Cheadle M A; Lindsay D S; Rowe S; Dykstra C C; Williams M A; Spencer J A; Toivio-Kinnucan M A; Lenz S D; Newton J C; Rolsma M D; Blagburn B L (Reprint)
AUTHOR ADDRESS: Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA**USA
JOURNAL: International Journal for Parasitology 29 (10): p1537-1543 Oct., 1999 1999
MEDIUM: print
ISSN: 0020-7519
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

...ABSTRACT: 100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). **Tachyzoites** were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the horse with CNS disease. **Tachyzoites** reacted with known N. caninum-positive serum from horses, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or equine anti-Sarcocystis **neurona** serum. Ultrastructural features of **tachyzoites** and results of comparison of **tachyzoite** immunodominant proteins revealed that they were identical to those of N. **hughesi** , a species described recently from a naturally infected horse. The isolate recovered from the naturally...

...horse in the present study (designated NA1) is thought to be an isolate of N. **hughesi** , although confirmation of this awaits additional molecular characterisation. These results provide some additional evidence that N. **hughesi** is a valid species and that Neospora infections in horses may occur in widely separated...

1/3,KWIC/15 (Item 1 from file: 50)
DIALOG(R)File 50:CAB Abstracts

0008366800 CAB Accession Number: 20033022389
Qualitative evaluation of selective tests for detection of Neospora hughesi antibodies in serum and cerebrospinal fluid of experimentally infected horses.
Packham, A. E.; Conrad, P. A.; Wilson, W. D.; Jeanes, L. V.; Sverlow, K. W.; Gardner, I. A.; Daft, B. M.; Marsh, A. E.; Blagburn, B. L.; Ferraro, G. L.; Barr, B. C.
Author email address: aepackham@ucdavis.edu
Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, One Shields Avenue, Davis,

CA 95616, USA.
Journal of Parasitology vol. 88 (6): p.1239-1246
Publication Year: 2002
ISSN: 0022-3395
Publisher: American Society of Parasitologists Lawrence, USA
Language: English Record Type: Abstract
Document Type: Journal article

Neospora **hughesi** is a newly recognized protozoan pathogen in horses that causes a myeloencephalitis similar to Sarcocystis **neurona** . There are no validated serological tests using the gold standard sera that are currently available to detect specific N. **hughesi** antibodies and, thus, no tests available to detect antemortem exposure or estimate seroprevalence in the...

... were to establish a bank of gold standard equine sera through experimental infections with N. **hughesi** and to assess several serological tests for the detection of related protozoan antibodies. Seven horses were inoculated with N. **hughesi** **tachyzoites** , and 7 horses received uninfected cell culture material. The horses were monitored, and blood and...

... indirect fluorescent antibody test. Qualitative and quantitative evaluation of the results showed that the N. **hughesi** indirect fluorescent antibody test (IFAT) consistently discriminated between experimentally infected and noninfected horses, using a...

... titers >1:640. Cerebrospinal fluid in all but one infected horse had very low N. **hughesi** IFAT titres (<1:160), starting at postinoculation day 30.

1/3,KWIC/16 (Item 2 from file: 50)
DIALOG(R)File 50:CAB Abstracts

0008241943 CAB Accession Number: 20023102736
Seroprevalence of Neospora , Toxoplasma gondii and Sarcocystis neurona antibodies in horses from Jeju island, South Korea.
Gupta, G. D.; Lakritz, J.; Kim JaeHoon; Kim DaeYong; Kim JinKap; Marsh, A. E.
Author email address: marshae@missouri.edu
Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Connaway Hall, 1600 East Rollins Dr., Columbia, MO 65211, USA.
Veterinary Parasitology vol. 106 (3): p.193-201
Publication Year: 2002
ISSN: 0304-4017
Digital Object Identifier: 10.1016/S0304-4017(02)00064-X
Publisher: Elsevier Science B.V. Amsterdam, Netherlands
Language: English Record Type: Abstract
Document Type: Journal article

... old) from Jeju island, South Korea [date not given]. For comparison, a naturally infected Neospora **hughesi** horse and an experimentally inoculated T. gondii equid (pony) were used. In addition, all samples were tested for antibodies to Sarcocystis **neurona** by immunoblot analysis. A total of 191 serum samples from clinically normal horses were evaluated...

... banding pattern of the positive control by immunoblot analysis. No sample was positive for N. **hughesi** by immunoblot analysis in this study. Overall, there was a 1% seroprevalence for T. gondii antibodies in the horses tested based on immunoblot analysis. The seroprevalence for S. **neurona** and N. **hughesi** antibodies was 0%. We concluded that these horses are either not routinely exposed to these...

... 3 years of age. This naive population of horses could be useful when evaluating S. **neurona** serodiagnostic tests or evaluating potential S. **neurona** **vaccines** since exposure risks to S. **neurona** and closely related parasites are negligible.

1/3,KWIC/17 (Item 3 from file: 50)
DIALOG(R)File 50:CAB Abstracts

0007835984 CAB Accession Number: 20000804749
Prevalence of antibodies to Neospora caninum in dogs [sic].
Cheadle, M. A.; Lindsay, D. S.; Rowe, S.; Dykstra, C. C.; Williams, M. A.; Spencer, J. A.; Toivio-Kinnucan, M. A.; Lenz, S. D.; Newton, J. C.; Rolsma, M. D.; Blagburn, B. L.
Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849, USA.
Conference Title: Neospora caninum and neosporosis.
International Journal for Parasitology vol. 29 (10): p.1537-1543
Publication Year: 1999
ISSN: 0020-7519
Digital Object Identifier: 10.1016/S0020-7519(99)00140-X
Language: English Record Type: Abstract
Document Type: Journal article

...100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). **Tachyzoites** were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the horse with CNS disease. The **tachyzoites** reacted with known N. caninum -positive serum from horses, cows, dogs and mice, but did not react with murine anti- Toxoplasma gondii or equine anti- Sarcocystis **neurona** serum. Ultrastructural features of the **tachyzoites** and a comparison of their immunodominant proteins showed that they were identical to those of N. **hughesi** . The isolate recovered from the horse in (designated NA1) is considered to be an isolate of N. **hughesi** , although additional molecular confirmation is required. The results support the recognition of N. **hughesi** as a valid species and show that Neospora infections in horses may occur in widely...

1/3,KWIC/18 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

11920051 EMBASE No: 2003030196
Qualitative evaluation of selective tests for detection of Neospora hughesi antibodies in serum and cerebrospinal fluid of experimentally infected horses
Packham A.E.; Conrad P.A.; Wilson W.D.; Jeanes L.V.; Sverlow K.W.; Gardner I.A.; Daft B.M.; Marsh A.E.; Blagburn B.L.; Ferraro G.L.; Barr B.C.

AUTHOR EMAIL: aepackham@ucdavis.edu
Journal of Parasitology (J. PARASITOL.) (United States) 2002, 88/6 (1239-1246)
CODEN: JOPAA ISSN: 0022-3395
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 42

Neospora **hughesi** is a newly recognized protozoan pathogen in horses that causes a myeloencephalitis similar to Sarcocystis **neurona** . There are no validated serologic tests using the gold standard sera that are currently available to detect specific N. **hughesi** antibodies and, thus, no tests available to detect antemortem exposure or estimate seroprevalence in the...
...were to establish a bank of gold standard equine sera through experimental infections with N. **hughesi** and to assess several serologic tests for the detection of related protozoan antibodies. Seven horses were inoculated with N. **hughesi** **tachyzoites** , and 7 horses received uninfected cell culture material. The horses were monitored, and blood and ...

...indirect fluorescent antibody test. Qualitative and quantitative evaluation of the results showed that the N. **hughesi** indirect fluorescent antibody test (IFAT) consistently discriminated between experimentally infected and noninfected horses, using a...

...titers >1:640. Cerebrospinal fluid in all but 1 infected horse had very low N. **hughesi** IFAT titers (<1:160), starting at postinoculation day 30.

1/3,KWIC/19 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE

11633355 EMBASE No: 2002204930
Seroprevalence of Neospora, Toxoplasma gondii and Sarcocystis neurona antibodies in horses from Jeju island, South Korea
Gupta G.D.; Lakritz J.; Kim J.-H.; Kim D.-Y.; Kim J.-K.; Marsh A.E.
A.E. Marsh, Dept. of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, 1600 East Rollins Dr., Columbia, MO 65211 United States
AUTHOR EMAIL: marshae@missouri.edu
Veterinary Parasitology (VET. PARASITOL.) (Netherlands) 26 JUN 2002, 106/3 (193-201)
CODEN: VPARD ISSN: 0304-4017
PUBLISHER ITEM IDENTIFIER: S030440170200064X
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 42

...Jeju island, South Korea (126degrees12prime E and 33degrees34prime N). For comparison, a naturally infected Neospora **hughesi** horse and an experimentally inoculated T. gondii equid (pony) were used. In addition, all samples were tested for antibodies to Sarcocystis **neurona** by immunoblot analysis. A total of 191 serum samples from clinically normal horses were evaluated...

...banding pattern of the positive control by immunoblot analysis. No

sample was positive for N. **hughesi** by immunoblot analysis in this study. Overall, there was a 1% seroprevalence for T. gondii antibodies in the horses tested based on immunoblot analysis. The seroprevalence for S. **neurona** and N. **hughesi** antibodies was 0%. We concluded that these horses are either not routinely exposed to these...

...3 years of age. This naive population of horses could be useful when evaluating S. **neurona** serodiagnostic tests or evaluating potential S. **neurona** **vaccines** since exposure risks to S. **neurona** and closely related parasites are negligible.

1/3,KWIC/20 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE

07918702 EMBASE No: 1999392491
Prevalence of antibodies to Neospora Caninum in dogs
Cheadle M.A.; Lindsay D.S.; Rowe S.; Dykstra C.C.; Williams M.A.; Spencer J.A.; Toivio-Kinnucan M.A.; Lenz S.D.; Newton J.C.; Rolsma M.D.; Blagburn B.L.
B.L. Blagburn, Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849 United States
AUTHOR EMAIL: blagbbl@vetmed.auburn.edu
International Journal for Parasitology (INT. J. PARASITOL.) (United Kingdom) 1999, 29/10 (1537-1543)
CODEN: IJPYB ISSN: 0020-7519
PUBLISHER ITEM IDENTIFIER: S002075199900140X
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 25

...100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). **Tachyzoites** were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the horse with CNS disease. **Tachyzoites** reacted with known N. caninum-positive serum from horses, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or equine anti-Sarcocystis **neurona** serum. Ultrastructural features of **tachyzoites** and results of comparison of **tachyzoite** immunodominant proteins revealed that they were identical to those of N. **hughesi** , a species described recently from a naturally infected horse. The isolate recovered from the naturally ...

...horse in the present study (designated NA1) is thought to be an isolate of N. **hughesi** , although confirmation of this awaits additional molecular characterisation. These results provide some additional evidence that N. **hughesi** is a valid species and that Neospora infections in horses may occur in widely separated...

1/3,KWIC/21 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

10098320 2002-0041886 2002-0011597
C/EQUINE PROTOZOAL MYELOENCEPHALITIS VACCINE; VETERINARY MEDICINE
Inventors: Bigbie Rocky Barry (US); Ng Terry Kaleung (US); Whalen Joseph

Wilson JR (US)
Assignee: Wyeth
Assignee Code: 03096

	Publication Number	Kind	Date	Application Number	Date
	US 20020041886	A1	20020411	US 2001840485	20010423
Priority Applic:				US 2001840485	20010423
Provisional Applic:				US 60-199435	20000425
				US 60-278695	20010326

Abstract: The present invention provides an immunogenically active component comprising inactivated Sarcocystis **neurona** cells and/ or inactivated Neospora **hughesi** cells; antigens derived therefrom; DNA derived therefrom; or a mixture; or in combination with other **vaccine** components thereof. Further provided are **vaccine** compositions useful for preventing or ameliorating equine protozoal myeloencephalitis infection and disease and a method...

Exemplary Claim: 1. An immunogenically active component which comprises a member selected from the group consisting of **merozoite** antibody inducing, inactivated Sarcocystis **neurona** cells; **tachyzoite** antibody inducing, inactivated Neospora **hughesi** cells; a **merozoite** or **tachyzoite** antibody inducing antigen derived from said cells; DNA derived from said cells capable of inducing a **merozoite** or **tachyzoite** antibody immune response; and a mixture thereof.

Non-exemplary Claims: ...13. The **vaccine** composition according to claim 12 wherein said adjuvant is a metabolizable oil...second immunogenically active component selected from the group consisting of tachyzoite antibody inducing, inactivated Neospora **hughesi** cells; a tachyzoite antibody inducing antigen derived from said cells; DNA derived from said cells...

...vaccine composition according to claim 15 wherein said first immunologically active component comprises inactivated Sarcocystis **neurona** cells and said second immunologically effective component comprises inactivated Neospora **hughesi** cells...

...which comprises a member selected from the group consisting of merozoite antibody inducing, inactivated Sarcocystis **neurona** cells; tachyzoite antibody inducing, inactivated Neospora **hughesi** cells; a merozoite or tachyzoite antibody inducing antigen derived from said cells; DNA derived from...

...which comprises a member selected from the group consisting of merezoite antibody inducing, inactivated Sarcocystis **neurona** cells; tachyzoite antibody inducing, inactivated Neospora **hughesi** cells; a merezoite or tachyzoite antibody inducing antigen derived from said cells; DNA derived from...

...first immunogenically active component selected from the group consisting of merozoite antibody inducing, inactivated Sarcocystis **neurona** cells; a merozoite antibody inducing antigen derived from said cells; DNA derived from said cells...

...second immunogenically active component selected from the group consisting of tachyzoite antibody inducing, inactivated Neospora

hughesi cells; a tachyzoite antibody inducing antigen derived from said cells; DNA derived from said cells capable of inducing a **tachyzoite** antibody immune response; or a mixture thereof; a pharmacologically acceptable carrier; and optionally an immunogenically...

...21. The method according to claim 18 wherein said **vaccine** is administered parenterally...

...22. The method according to claim 18 wherein said **vaccine** is administered intramuscularly...

...23. A method for the cell culture propagation of Sarcocystis **neurona** or Neospora **hughesi** protozoan parasite which comprises: a) growing a monolayer of cells having a confluency of 80%-100%; b) refeeding said cells with supplemented growth media; c) inoculating said cells with **merozoites** or **tachyzoites** ; d) holding the inoculated cells for 4-12 days; e) decanting the supplemented growth media...

1/3,KWIC/22 (Item 2 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

04116589 2004-0027835
C/(A1) ANIMAL MODEL FOR INFECTION BY AN APICOMPLEXAN PARASITE; PARASITICIDES; CENTRAL NERVOUS SYSTEM DISORDERS; THERAPY, VACCINES, DIAGNOSIS
(B2) ANIMAL MODEL FOR INFECTION BY AN APICOMPLEXAN PARASITE; PARASITICIDES; CENTRAL NERVOUS SYSTEM DISORDERS; THERAPY, VACCINES, DIAGNOSIS
Inventors: Ellison Siobhan P (US)
Assignee: (A1) Unassigned Or Assigned To Individual
(B2) Unassigned Or Assigned To Individual
Assignee Code: (A1) 68000; (B2) 68000

	Publication Number	Kind	Date	Application Number	Date
	US 20030219381	A1	20031127	US 2002152960	20020522
	US 6780415	B2	20040824	US 2002152960	20020522
Prior Publication:	US 20030219381	A1	20031127		
Priority Applic:				US 2002152960	20020522
Provisional Applic:				US 60-302007	20010702

Calculated Expiration: 20220522
...Division Pub(No),Applic(No,Date): 30. A **vaccine** composition for protecting against Apicomplexan parasite diseases comprising components that target activated lymphocytes to produce...

...method of claim 1 wherein the Apicomplexan parasite is selected from Sarcocystis dasypus (syn. S. **neurona**), Sarcocystis **neurona** , Sarcocystis falcatula, Toxoplasma gondil, Neospora caninum, Neospora **hughesi** , Samocystis cruzi, Sarcocystis spp., Eimeria spp. and Plasmodium spp...

...1, wherein the infected susceptible homologous cell consists of a leukocyte comprising at least a **merozoite** stage of a parasite selected from Sarcocystis **neurona** , Sarcocystis falcatula, and Neurosporosis **hughesi** , said **merozoite** having an unextruded conoid...

1/3,KWIC/23 (Item 1 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00854074 **Image available**
DECOQUINATE, 4-HYDROXYQUINOLONES AND NAPTHOQUINONES FOR THE PREVENTION AND TREATMENT OF EQUINE PROTOZOAL MYELOENCEPHALITIS CAUSED BY SARCOCYSTIS NEURONA HUGHESI AND OTHER APICOMPLEXAN PROTOZOANS
DECOQUINATE, 4-HYDROXYQUINOLONES ET NAPTHOQUINONES DESTINES A LA PREVENTION ET AU TRAITEMENT DE LA MYELOENCEPHALITE EQUINE A PROTOZOAIRE CAUSEE PAR <I>SARCOCYSTIS NEURONA</I>, <I>NEOSPORA HUGHESI</I> ET AUTRES PROTOZOAIRES APICOMPLEXES
Patent Applicant/Assignee:
VIRGINIA TECH INTELLECTUAL PROPERTIES INC, 1872 Pratt Drive, Suite 1625, Blacksburg, VA 24060, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
LINDSAY David S, 1655 Sleepyhollow Road, Christiansburg, VA 24073, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
WHITHAM Michael E (et al) (agent), McGuireWoods, LLP, Suite 1800, 1750 Tysons Boulevard, McLean, VA 22102, US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200187290 A1 20011122 (WO 0187290)
Application: WO 2001US15931 20010517 (PCT/WO US0115931)
Priority Application: US 2000204771 20000517
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 3580

Fulltext Availability:
Detailed Description

Detailed Description
... agent may be administered orally as part of feed, or by other means such
as **injection** .

Regular (such as daily) feeding of the above-mentioned anti EPM compounds to horses may have flirther beneficial effects, such as (1) prevention of abortions due to Neospora caninum, Neospora **hughesi** or Toxoplasma gondii; (2) prevention of equine babesiosis (because the piroplasmas have mitochondria that are...

...by Eimeria leuckarti in foals and other equids.

The following experimentation was conducted for Sarcocystis **neurona** isolates and cell culture.

Sarcocystis **neurona** **merozoites** (SN2, SN3, or SN6 strains, isolated from a horse with EPM (Dubey et al., 2001 of diclazuril against Sarcocystis **neurona** and Sarcocystisfalcatula in cell cultures, J. Parasitol. 86, 164-166 (2000). The host cells were...

...37 C in a humidified atmosphere containing 5% C02 and 95% air.

, For quantitative studies, **merozoites** were harvested from infected cell cultures by removing the medium and replacing it with Hanks...

...These results demonstrated that decoquinate can effectively inhibit several strains of Sarcocystis neurona.

Experiment 3. **Merozoites** of the SN3 strain were inoculated on to cell cultures and allowed to penetrate host...

...or 0.0001 (2 flasks) microgram/ml decoquinate continuously for 10 days.

Control flasks contained **merozoites** but no decoquinate. The numbers of **merozoites** produced were detertnined at 1 0 days and a percent reduction in **merozoite** production deten-nined. Treatment with 0.01 microgram/ml caused a 98% reduction in **merozoite** production.

Treatment with 0.001 microgram/ml caused a 87% reduction in **merozoite** production. Treatment with 0. 000 I microgram/ml caused a 40% reduction in **merozoite** production. These findings indicate that there is a dose response to decoquinate and suggests setting...
...view of EPM being a neurologic syndrome in horses caused primarily by infection with Sarcocystis **neurona** and rarely with Neospora **hughesi** , and further considering that EPM is the most important protozoal disease of horses in the...

...The experimental data set forth above indicate that decoquinate can quickly kill stages of Sarcocystis **neurona** in cell cultures. Decoquinate also exerts its anti-Sarcocystis **neurona** activity at low doses in cell cultures, and is safe in the horse
and not...Horses on preventative decoquinate treatment are expected to be protected against EPM caused by Neospora **hughesi** , as delivery systems that are preventative against Sarcocystis **neurona** will be preventative against Neospora **hughesi** .

Again without limiting the invention to such an example, an example of treating EPM would...

1/3,KWIC/24 (Item 2 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00849074
EQUINE PROTOZOAL MYELOENCEPHALITIS VACCINE
VACCIN CONTRE LA MYELOENCEPHALITE PROTOZOAIRE DU CHEVAL

Patent Applicant/Assignee:
AMERICAN HOME PRODUCTS CORPORATION, Five Giralda Farms, Madison, NJ 07940 , US, US (Residence), US (Nationality)
Inventor(s):
BIGBIE Rocky Barry, 20195 Countryview Drive, Spring Hill, KS 66083, US,
NG Terry Kaleung, 2027 North 30th Court, Fort Dodge, IA 50501, US,
WHALEN Joseph Wilson Jr, 520 South 15th Street, Fort Dodge, IA 50501, US,

Legal Representative:
MANDEL Adley F (agent), American Home Products Corporation, Patent Law Dept., Five Giralda Farms, Madison, NJ 07940 (et al), US,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200180885 A2-A3 20011101 (WO 0180885)
Application: WO 2001US40527 20010413 (PCT/WO US0140527)
Priority Application: US 2000199435 20000425; US 2001278695 20010326
Designated States:
(Protection type is "patent" unless otherwise stated - for applications prior to 2004)
AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ
EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ
TM TR TT TZ UA UG UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 5876

Fulltext Availability:
Detailed Description
Claims

English Abstract
The present invention provides an immunogenically active component comprising inactivated Sarcocystis **neurona** cells and/or inactivated Neospora **hughesi** cells; antigens derived therefrom; DNA derived therefrom; or a mixture; or in combination with other **vaccine** components thereof. Further provided are **vaccine** compositions useful for preventing or ameliorating equine protozoal myeloencephalitis infection and disease and a method...

French Abstract
La presente invention concerne un composant immunogenetiquement actif comprenant des cellules inactivees de Sarcocystis **neurona** et/ou des cellules inactivees de Neospora **hughesi** , des antigenes derives de ces cellules, de l'ADN derive de ces cellules, ou un melange, ou en combinaison avec d'autres composants **vaccinaux** en provenant. L'invention concerne egalement des compositions **vaccinales** permettant de prevenir ou d'ameliorer des infections et affections liees a la myeloencephalite protozoaire...

Detailed Description
EQUINE PROTOZOAL MYELOENCEPHALITIS **VACCINE**
ABSTRACT OF THE INVENTION
The present invention provides an immunogenically active component comprising inactivated Sarcocystis...

...group consisting of merozoite antibody inducing, inactivated Sarcocystis neurona cells; tachyzoite antibody inducing, inactivated lVeospora **hughesi** cells; a merozoite or tachyzoite antibody inducing antigen derived from said cells; DNA derived from...parasites, including Sarcocystis spp. and Neospora spp.

DETAILED DESCRIPTION OF THE INVENTION

Sa.rcoc.ystis **neurona** or Neospo-ra **hughesi** protozoa are the causative agents of equine protozoal myeloencephalitis (EPM) disease, which is a serious...

...host and apparently become infected when ingesting feed which has been contaminated with the Sarcocystis **neurona** or Neospora **hughesi** protozoans via opossum fecal contamination. EPM disease when untreated will progress from initial numbness of...

...Surprisingly, it has now been found that an immunogenically active component which comprises inactivated Sarcocystis **neurona** cells or antigens, subunit proteins or plasmid DNA; inactivated Neospora 5 **hughesi** cells or antigens, subunit proteins or plasmid DNA; or mixtures thereof may be administered in...

...composition to prevent or ameliorate EPM disease in equines, particularly horses. Antigens derived from Sarcocystis **neurona** or Neospora **hughesi** may be obtained using conventional procedures such as outer membrane extraction. Plasmid DNA derived from Sarcoc.ystis **neurona** or Neospora **hughesi** may be obtained via isolation from sources such as the fluids or tissues of equine...

...A useful starting isolate for the vaccines of the invention include, for example, for Sarcocystis **neurona** , the isolate designated SN3; other such isolates are those known as SN1, SN2, SN4, SN5...

...Oregon State University, the University of Missouri and others. A culture of one such Sarcocystis **neurona** isolate designated SN9, originally isolated from the intestinal scrapings of the opossum and confirmed to be a representative Sarcocystis **neurona** by PCR, was deposited with the ATCC on January 25, 2001, and given ATCC Accession...

...A useful starting isolate for the vaccines of the invention include, for example, for Neospora **hughesi** , the isolate designated NEQ1; another such isolate is that known as NE1, which has been...

...growth media; refeeding the cells with fresh growth media; inoculating the cells with merozoites or **tachyzoites** ; after 4-12 days, decanting the growth media; and refeeding the inoculated cells a second...Sa-rcocystis neurona

merozoites, resulting in a final serum dilution of 1:4. The organism (**merozoite**) levels used 1:10 are 2.5x105, 1:100 are 2.5xl 04 , and 1:1000 are 2. SxlO' **merozoites** per mL. Duplicate sets of serum/organism tubes are set up using a serum pool from the group 4 non- **vaccinated** horses to stand as a negative control group for comparison. The 2.OmL organism/serum...

...of plaques observed in the flasks which had received the serum from the group 3 **vaccinate** horses which had been incubated with organism at all organism dilutions when compared to similar flasks which had the non- **vaccinated** control serum. This data is shown in Table II below.

As can be seen from the data in Table II, the degree of plaque reduction in every case of the **vaccinated** horse serum pools exceeded 70%.

TABLE II

Sarcocystis **neurona** Placrue Reduction Serolocry

Organism Dilution	Serum Sample	Plaques Average No, Percent Dilution	Observed	of Placrues	Reduction
1:10	**Vaccine**	3 1:4	87	97.0	89.22
1:10	**Vaccine**	3 1:4	107		
1:100	**Vaccine**	3 1:4	16	14.0	73.33
1:100	**Vaccine**	3 1:4	12		
1:1000	**Vaccine**	3 1:4	2	1.5	85.00
1:1000	**Vaccine**	3 1:4	1		
1:10	Controls	1:4	TNTC	TNTC	NA
1:10	Controls...				

...to the number of plaques in the corresponding control serum dilution plaque count.

EXAMPLE 4

Vaccine -preparation

Meospora **hughesi** is obtained from the brain or spinal column of a horse that has been diagnosed...

...cultures of E. Derm or Vero cells in RPMI tissue culture medium at 370C. The **tachyzoites** harvested are counted at the time of harvest and then inactivated by means of addition...

Claim

1 An immunogenically active component which comprises a member selected from the group consisting of **merozoite** antibody inducing, inactivated Sarcocystis neurona cells; tachyzoite antibody inducing, inactivated Neospora hughesi cells; a merozoite...

...first immunogenically active component selected from the group consisting of merozoite antibody inducing,

inactivated Sarcocystis **neurona** cells; a merozoite antibody inducing antigen derived from said cells; DNA derived from said cells...

...second immunogenically active component selected from the group consisting of tachyzoite antibody inducing, inactivated Neospora **hughesi** cells; a tachyzoite antibody inducing antigen derived from said cells; DNA derived from said cells...

...vaccine composition according to claim 15 wherein said first immunologically active component comprises inactivated Sarcocystis **neurona** cells and said second immunologically effective component comprises inactivated Neospora **hughesi** cells.

17 The vaccine composition according to claim 15 wherein said first immunologically active component...

...which comprises a member selected from the group consisting of merozoite antibody inducing, inactivated Sarcocystis **neurona** cells; tachyzoite antibody inducing, inactivated Neospora **hughesi** cells; a merozoite or tachyzoite antibody inducing antigen derived from said cells; DNA derived from...

...which comprises a member selected from the group consisting of merezoite antibody inducing, inactivated Sarcocystis **neurona** cells; tachyzoite antibody inducing, inactivated Neospora **hughesi** cells; a merezoite or tachyzoite antibody inducing antigen derived from said cells; DNA derived from...

...first immunogenically active component selected from the group consisting of merozoite antibody inducing, inactivated Sarcocystis **neurona** cells; a merozoite antibody inducing antigen derived from said cells; DNA derived from said cells...

...second immunogenically active component selected from the group consisting of tachyzoite antibody inducing, inactivated Neospora **hughesi** cells; a tachyzoite antibody inducing antigen derived from said cells; DNA derived from said cells...

...and optionally an immunogenically stimulating adjuvant.

21 The method according to claim 18 wherein said **vaccine** is administered parenterally.

22 The method according to claim 18 wherein said **vaccine** is administered intramuscularly.

23 A method for the cell culture propagation of Sarcocystis **neurona** or Neospora **hughesi** protozoan parasite which comprises:
a) growing a monolayer of cells having a confluency of 80%-100%;
b) refeeding said cells with supplemented growth media;

naturally-, experimentally-, and **vaccinated** horses for the diagnosis of EPM caused by <italic>S. **neurona** </italic>. Using the IFAT, the risk of transplacental transmission and the risk and age at first exposure to <italic>S. **neurona** </italic> and <italic> N. **hughesi** </italic> in a cohort of mares (n = 337) and foals (n = 484) in 4 California...

...of transplacental transmission of either parasite. The risk of post-natal exposure to <italic>S. **neurona** </italic> and <italic>N. **hughesi** </italic> was 8.2% and 3.1% respectively, over the study period (2.5 yrs). There was a significant difference in the risk of exposure to <italic>S. **neurona** </italic> among farms (<italic>P</italic> = 0.005) but not in the risk of exposure to <italic>N. **hughesi** </italic> (<italic>P</italic> = 0.83). The median age at first exposure was 1.2 yr for <italic>S. **neurona** </italic> and 0.8 yr for <italic>N. **hughesi** </italic>. The annual incidence rate of EPM among mares and foals were 0.2% and...

...CSF, and has potential for use in the diagnosis of EPM caused by <italic> S. **neurona** </italic>. There was no detectable risk of transplacental transmission of <italic>S. **neurona** </italic> and <italic>N. **hughesi** </italic>, and the risk of exposure to either parasite was low between birth and 2...

1/3,KWIC/27 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

15973467 PASCAL No.: 03-0117696

Qualitative evaluation of selective tests for detection of Neospora hughesi antibodies in serum and cerebrospinal fluid of experimentally infected horses

PACKHAM Andrea E; CONRAD Patricia A; WILSON W David; JEANES Lisa V; SVERLOW Karen W; GARDNER Ian A; DAFTT Barbara M; MARSH Antoinette E; BLAGBURN Byron L; FERRARO Gregory L; BARR Bradd C

Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, California 95616, United States

Journal: The Journal of parasitology, 2002, 88 (6) 1239-1246

Language: English

Neospora **hughesi** is a newly recognized protozoan pathogen in horses that causes a myeloencephalitis similar to Sarcocystis **neurona** . There are no validated serologic tests using the gold standard sera that are currently available to detect specific N. **hughesi** antibodies and, thus, no tests available to detect antemortem exposure or estimate seroprevalence in the...
... were to establish a bank of gold standard equine sera through experimental infections with N. **hughesi** and to assess several serologic tests for the detection of related protozoan antibodies. Seven horses were inoculated with N. **hughesi** **tachyzoites** , and 7 horses received uninfected cell culture material. The horses were monitored, and blood and ...

... indirect fluorescent antibody test. Qualitative and quantitative evaluation of the results showed that the N. **hughesi** indirect fluorescent

antibody test (IFAT) consistently discriminated between experimentally infected and noninfected horses, using a...

... titers >1:640. Cerebrospinal fluid in all but 1 infected horse had very low N. **hughesi** IFAT titers (<1:160), starting at postinoculation day 30.

1/3,KWIC/28 (Item 1 from file: 342)
DIALOG(R)File 342:Derwent Patents Citation Indx

04947323 WPI Acc No: 02-049244/06
Vaccine useful for preventing or ameliorating equine protozoal myeloencephalitis disease, comprises inactivated Sarcocystis neurona cells and/or Neospora hughesi cells, antigens, DNA derived from the cells or their mixtures -
Patent Assignee: (AMHP) AMERICAN HOME PROD CORP
Author (Inventor): BIGBIE R B; NG T K; WHALEN J W
Patent (basic)

Patent No	Kind	Date	Examiner	Field of Search
WO 200180885	A2	011101 (BASIC)		

Derwent Week (Basic): 0206
Priority Data: US 199435P (000425); US 278695P (010326)
Applications: AU 200151761 (010413); BR 200110232 (010413); EP 2001925175 (010413); WO 2001US40527 (010413); US 840485 (010423)
Designated States
(National): AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; BZ; CA; CH; CN; CO; CR; CU; CZ; DE; DK; DM; DZ; EE; ES; FI; GB; GD; GE; GH; GM; HR ; HU; ID; IL; IN; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA; MD; MG; MK; MN; MW; MX; MZ; NO; NZ; PL; PT; RO; RU; SD; SE; SG ; SI; SK; SL; TJ; TM; TR; TT; TZ; UA; UG; UZ; VN; YU; ZA; ZW
(Regional): AL; AT; BE; CH; CY; DE; DK; EA; ES; FI; FR; GB; GH; GM; GR; IE; IT; KE; LI; LS; LT; LU; LV; MC; MK; MW; MZ; NL; OA; PT; RO; SD; SE ; SI; SL; SZ; TR; TZ; UG; ZW
Derwent Class: B04; C06; D16
Int Pat Class: A61K-039/02; A61K-039/395; A61K-048/00; A61P-033/00; C07K-016/20
Number of Patents: 005
Number of Countries: 096
Number of Cited Patents: 002
Number of Cited Literature References: 004
Number of Citing Patents: 000

1/3,KWIC/29 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0280356 DBR Accession No.: 2002-04497 PATENT
Vaccine useful for preventing or ameliorating equine protozoal myeloencephalitis disease, comprises inactivated Sarcocystis neuroma cells and/or Neospora hughesi cells, antigens, DNA derived from the cells or their mixtures - horse protozoon myeloencephalitis disease therapy suing a recombinant vaccine or a nucleic acid vaccine
AUTHOR: Bigbie R B; Ng T K; Whalen Jr J W
CORPORATE SOURCE: Madison, NJ, USA.
PATENT ASSIGNEE: American-Home-Prod. 2001
PATENT NUMBER: WO 200180885 PATENT DATE: 20011101 WPI ACCESSION NO.:

2002-049244 (200206)
PRIORITY APPLIC. NO.: US 278695 APPLIC. DATE: 20010326
NATIONAL APPLIC. NO.: WO 2001US40527 APPLIC. DATE: 20010413
LANGUAGE: English

ABSTRACT: An immunogenically active component (I) having a **merozoite** antibody inducing, inactivated Sarcocystis **neurona** cells, **tachyzoite** antibody inducing, inactivated Neospora **hughesi** cells, a **merozoite** or **tachyzoite** antibody inducing antigen derived from the cells, DNA derived from the cells capable of inducing a **merozoite** or **tachyzoite** antibody immune response or their mixture, is new. Also claimed are: a **vaccine** composition comprising (I), a pharmacologically acceptable carrier, and an immunogenically stimulating adjuvant; cell culture propagation (III) of S. **neurona** or N. **hughesi** protozoan parasite, involving growing a monolayer of cells, re-feeding with supplemented growth medium, inoculating with **merozoites** or **tachyzoites** , holding for 4-12 days and decanting the supplemented growth medium from the inoculated cells...
DESCRIPTORS: **merozoite** antibody inducing Sarcocystis **neurona** cell, **tachyzoite** antibody inducing Neospora **hughesi** cell, antigen, appl. horse protozoon myeloencephalitis disease therapy, horse dermal, cattle kidney, African green monkey...

...mouse monocyte, fetal rhesus monkey kidney, feline kidney, dog kidney, hamster kidney cell, nucleic acid **vaccine** , recombinant **vaccine** mammal animal (Vol.21, No.8)

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  File 654:US Pat.Full. 1976-2005/Jun 09
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         (c) 2005 INIST/CNRS
  File 342:Derwent Patents Citation Indx 1978-05/200536
         (c) 2005 Thomson Derwent
  File 357:Derwent Biotech Res. _1982-2005/Jun W1
         (c) 2005 Thomson Derwent & ISI
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Terminal set to DLINK
? e sarcocystis
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Ref   Items   RT  Index-term
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E2        1       SARCOCYSTIQUE
E3     1390    3 *SARCOCYSTIS
E4       19       SARCOCYSTIS --ANALYSIS --AN
E5        3       SARCOCYSTIS --ANATOMY AND HISTOLOGY --AH
E6        5       SARCOCYSTIS --CHEMISTRY --CH
E7      146       SARCOCYSTIS --CLASSIFICATION --CL
E8       58       SARCOCYSTIS --CYTOLOGY --CY
E9       13       SARCOCYSTIS --DRUG EFFECTS --DE
E10      19       SARCOCYSTIS --ENZYMOLOGY --EN
E11      86       SARCOCYSTIS --GENETICS --GE
E12     228       SARCOCYSTIS --GROWTH AND DEVELOPMENT --GD

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--isolation and purification--IP; Toxoplasmosis, Animal--epidemiology--EP
CAS Registry No.: 0 (Antibodies, Protozoan)
Record Date Created: 19991015
Record Date Completed: 19991015

5/9/3
DIALOG(R)File 155:MEDLINE(R)

13165399 PMID: 11155929
The South American opossum, Didelphis marsupialis, from Brazil as another definitive host for Sarcocystis **speeri Dubey and Lindsay, 1999.**
Dubey J P; Kerber C E; Lindsay D S; Kasai N; Pena H F
United States Department of Agriculture, Agricultural Research Service, Livestock and Poultry Sciences Institute, Beltsville, Maryland 20705-2350, USA. jdubey@lpsi.barc.usda.gov
Parasitology (England) Dec 2000, 121 Pt 6 p589-94, ISSN 0031-1820 Journal Code: 0401121
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The North American opossum, Didelphis virginiana, is a definitive host for at least 3 species of Sarcocvstis: S. falcatula Stiles 1983, S. **neurona** Dubey, Davis, Speer, Bowman, de Lahunta, Granstrom, Topper, Hamir, Cummings, Suter 1991, and S. speeri Dubey and Lindsay 1999. In order to identify species of **Sarcocystis** in the South American opossum, D. inarsupialis, **Sarcocystis** sporocysts from the intestines of a naturally infected opossum (D. marsupialis) from Brazil were fed to 4 gamma-interferon knockout (KO) mice, a nude mouse, and 2 budgerigars (Melopsittacus undulatus). All 4 KO mice became ill and 1 died 42 days post-feeding (p.f.) of sporocysts, 1 was killed 44 days p.f. because of neurological signs, and 2 were killed 52 and 53 days p.f. because of abnormal gaits. Numerous sarcocysts were seen in the skeletal muscles of all 4 KO mice and they were structurally identical to S. speeri seen in KO mice fed sporocysts from D. virginiana from the United States and D. albiventris from Argentina. The nude mouse was killed 41 days p.f. because it appeared weak; schizonts were seen in sections of its liver and sarcocysts were seen in sections of skeletal muscles. **Sarcocystis** speeri was cultured in bovine turbinate cells inoculated with liver homogenate from this mouse. **Sarcocystis neurona** was not demonstrable in tissues of mice. The two budgerigars remained asymptomatic and S. falcatula was not found in their tissues when they were killed 29 days p.i. This is the first report of S. speeri from D. marsupialis.
Descriptors: *Opossums--parasitology--PS; * **Sarcocystis** --physiology--PH; *Sarcocystosis--veterinary--VE; Animals; Bird Diseases--immunology--IM; Bird Diseases--parasitology--PS; Brain--parasitology--PS; Brazil; Gait; Host-Parasite Relations; **Immunity** , Natural; Liver--parasitology--PS; Mice ; Mice, Inbred C57BL; Mice, Knockout; Mice, Nude; Microscopy, Electron; Muscle, Skeletal--parasitology--PS; Parrots--parasitology--PS; **Sarcocystis** --classification--CL; **Sarcocystis** --ultrastructure--UL; Sarcocystosis --pathology--PA
Record Date Created: 20010111
Record Date Completed: 20010315

5/9/4
DIALOG(R)File 155:MEDLINE(R)

13151015 PMID: 11128499
Immunohistochemical confirmation of Sarcocystis neurona **infections in raccoons, mink, cat, skunk, and pony.**
Dubey J P; Hamir A N
Parasite Biology and Epidemiology Laboratory, Livestock and Poultry Sciences Institute, ARS, USDA, Beltsville, Maryland 20705, USA.
Journal of parasitology (United States) Oct 2000, 86 (5) p1150-2, ISSN 0022-3395 Journal Code: 7803124
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
In the central nervous system of 2 raccoons, 1 cat, 1 pony, 2 mink, and 1 skunk, protozoa previously thought to be **Sarcocystis** -like reacted positively to **Sarcocystis neurona** -specific antibodies in an immunohistochemical test. In addition, S. **neurona** was identified in the brain of another skunk. These observations indicate that S. **neurona** is not confined to opossums and horses.
Descriptors: *Carnivora--parasitology--PS; *Horse Diseases--parasitology--PS; * **Sarcocystis** --isolation and purification--IP; *Sarcocystosis--veterinary--VE; Animals; Antibodies, Protozoan--immunology--IM; **Antigens**, Protozoan--analysis--AN; Cat Diseases--diagnosis--DI; Cat Diseases--parasitology--PS; Cats; Horse Diseases--diagnosis--DI; Horses--parasitology--PS; Immunohistochemistry; Mephitidae--parasitology--PS; Mink--parasitology--PS; Rabbits; Raccoons--parasitology--PS; **Sarcocystis**--immunology--IM; Sarcocystosis--diagnosis--DI; Sarcocystosis--parasitology--PS
CAS Registry No.: 0 (Antibodies, Protozoan); 0 (Antigens, Protozoan)
Record Date Created: 20001220
Record Date Completed: 20010111

5/9/5
DIALOG(R)File 155:MEDLINE(R)

12999317 PMID: 10958438
In vitro cultivation of schizonts of Sarcocystis **speeri Dubey and Lindsay, 1999.**
Dubey J P; Speer C A; Lindsay D S
United States Department of Agriculture, Agricultural Research Service, Livestock and Poultry Sciences Institute, Beltsville, Agricultural Research Center, Maryland 20705-2350, USA.
Journal of parasitology (UNITED STATES) Aug 2000, 86 (4) p671-8, ISSN 0022-3395 Journal Code: 7803124
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Schizonts of **Sarcocystis** speeri Dubey and Lindsay, 1999 were cultured in vitro in bovine monocyte and equine kidney cell cultures inoculated with infected tissues of nude and gamma-interferon knockout mice fed sporocysts from opossums, Didelphis albiventris. At least 1 asexual cycle was completed in 3 days. In vitro-grown merozoites were structurally and **antigenically** distinct from those of **Sarcocystis neurona** and **Sarcocystis** falcatula. Culture-derived merozoites of S. speeri were not infective to budgerigars (Melopsittacus undulatus).

Descriptors: ***Sarcocystis** --growth and development--GD; *Sarcocystosis --parasitology--PS; Animals; Brain--parasitology--PS; Cattle; Cell Line; Cercopithecus aethiops; Histocytochemistry; Horses; Immunohistochemistry; Kidney--cytology--CY; Kidney--parasitology--PS; Liver--parasitology--PS; Mice; Mice, Knockout; Mice, Nude; Microscopy, Phase-Contrast; Monocytes --cytology--CY; Monocytes--parasitology--PS; Opossums--parasitology--PS; Parrots; **Sarcocystis** --pathogenicity--PY

Record Date Created: 20000906

Record Date Completed: 20000906

5/9/6

DIALOG(R)File 155:MEDLINE(R)

12992935 PMID: 10946139

Inoculation of Sarcocystis neurona **merozoites into the central nervous system of horses.**

Lindsay D S; Dykstra C C; Williams A; Spencer J A; Lenz S D; Palma K; Dubey J P; Blagburn B L

Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, 24061-0342, USA. lindsayd@vt.edu

Veterinary parasitology (NETHERLANDS) Sep 20 2000, 92 (2) p157-63, ISSN 0304-4017 Journal Code: 7602745

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Equine protozoal myeloencephalitis (EPM) is a neurologic syndrome in horses from the Americas and is usually caused by infection with the apicomplexan parasite, **Sarcocystis neurona** . A horse model of EPM is needed to test the efficacy of chemotherapeutic agents and potential **vaccines** . Five horses that were negative for antibodies to S. **neurona** in their serum and cerebrospinal fluid (CSF) were injected in the subarachnoid space with living merozoites of the SN2 isolate of S. **neurona** . None of the horses developed clinical disease or died over a 132-day observation period. All five horses developed antibodies to S. **neurona** in their CSF and serum 3-4 weeks after injection. Two of the horses were examined at necropsy and no parasite induced lesions were observed in their tissues and no parasites were recovered from portions of their spinal cords inoculated on to cell cultures. Results of this study demonstrate that merozoites of the SN2 isolate of S. **neurona** will induce seroconversion but not clinical disease when inoculated directly into the CSF of nonimmune horses.

Tags: Female; Male; Research Support, Non-U.S. Gov't

Descriptors: *Encephalomyelitis--veterinary--VE; *Horse Diseases --parasitology--PS; * **Sarcocystis** --pathogenicity--PY; *Sarcocystosis

Tags: Female; Research Support, Non-U.S. Gov't
Descriptors: *Antibodies, Protozoan--blood--BL; *Coccidiosis--veterinary --VE; *Horse Diseases--epidemiology--EP; *Neospora--immunology--IM; *Neospora--isolation and purification--IP; Animals; Antibodies, Protozoan --immunology--IM; Cattle; Coccidiosis--epidemiology--EP; Coccidiosis --parasitology--PS; Dogs; Fluorescent Antibody Technique, Indirect; Horse Diseases--parasitology--PS; Horses; Mice; Myelitis--parasitology--PS; Myelitis--veterinary--VE; Neospora--ultrastructure--UL; Prevalence; Spinal Cord--parasitology--PS
CAS Registry No.: 0 (Antibodies, Protozoan)
Record Date Created: 20000120
Record Date Completed: 20000120

5/9/9
DIALOG(R)File 155:MEDLINE(R)

12656705 PMID: 10577729
Sarcocystis **speeri N. sp. (Protozoa:** Sarcocystidae **) from the opossum (Didelphis virginiana).**
Dubey J P; Lindsay D S
U.S. Department of Agriculture, Agricultural Research Service, Livestock and Poultry Sciences Institute, Beltsville Agricultural Research Center, Maryland 20705-2350, USA.
Journal of parasitology (UNITED STATES) Oct 1999, 85 (5) p903-9, ISSN 0022-3395 Journal Code: 7803124
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The North American opossum (Didelphis virginiana) is host to at least 3 species of **Sarcocystis** : Sarcocystisfalcatula, **Sarcocystis neurona** , and a recently recognized **Sarcocystis** sp. A new name, **Sarcocystis** speeri, is proposed for the third unnamed **Sarcocystis** . Immunodeficient mice are an experimental intermediate host for S. speeri. **Sarcocystis** speeri sporocysts are 12-15 x 8-10 microm in size, and its schizonts are found in many organs of mice. Sarcocysts of S. speeri are found in skeletal muscles and they are up to 5 mm long and filiform. By light microscopy, the sarcocyst wall is thin (<1 microm thick); ultrastructurally, the cyst wall is up to 1.8 microm thick and has characteristic steeple-shaped villar protrusions surmounted by a spire. **Sarcocystis** speeri schizonts are morphologically and **antigenically** distinct from schizonts of S. **neurona** , and S. speeri sporocysts were not infective to budgerigars (Melopsittacus undulatus).
Descriptors: *Intestinal Diseases, Parasitic--veterinary--VE; *Opossums --parasitology--PS; * **Sarcocystis** --classification--CL; *Sarcocystosis --veterinary--VE; Animals; Immunohistochemistry; Intestinal Diseases, Parasitic--parasitology--PS; Intestine, Small--parasitology--PS; Liver --parasitology--PS; Lung--parasitology--PS; Mice; Mice, Knockout; Mice, Nude; Microscopy, Electron; Muscle, Skeletal--parasitology--PS; Parrots; Rabbits; **Sarcocystis** --immunology--IM; **Sarcocystis** --ultrastructure--UL; Sarcocystosis--parasitology--PS; Spleen--parasitology--PS
Record Date Created: 19991202
Record Date Completed: 19991202

Liang F T; Granstrom D E; Zhao X M; Timoney J F
Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington 40546-0099, USA.
Infection and immunity (UNITED STATES) May 1998, 66 (5) p1834-8, ISSN 0019-9567 Journal Code: 0246127
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Sarcocystis neurona is the etiologic agent of equine protozoal myeloencephalitis (EPM). Based on an analysis of 25,000 equine serum and cerebrospinal fluid (CSF) samples, including samples from horses with neurologic signs typical of EPM or with histologically or parasitologically confirmed EPM, four major immunoblot band patterns have been identified. Twenty-three serum and CSF samples representing each of the four immunoblot patterns were selected from 220 samples from horses with neurologic signs resembling EPM and examined for inhibitory effects on the infectivity of S. **neurona** by an in vitro neutralization assay. A high correlation between immunoblot band pattern and neutralizing activity was detected. Two **proteins**, Sn14 and Sn16 (14 and 16 kDa, respectively), appeared to be important for in vitro infection. A combination of the results of surface **protein** labeling, immunoprecipitation, Western blotting, and trypsin digestion suggests that these molecules are surface **proteins** and may be useful components of a **vaccine** against S. **neurona** infection. Although S. **neurona** is an obligate intracellular parasite, it is potentially a target for specific antibodies which may lyse merozoites via complement or inhibit their attachment and penetration to host cells.

Tags: Research Support, Non-U.S. Gov't
Descriptors: *Encephalitis--veterinary--VE; *Horse Diseases--immunology --IM; *Protozoan **Proteins** --immunology--IM; * **Sarcocystis** --immunology--IM ; *Sarcocystosis--veterinary--VE; Animals; Antibodies, Protozoan --immunology--IM; Encephalitis--immunology--IM; Horses; Immunoblotting; Membrane **Proteins** --immunology--IM; Neutralization Tests; Precipitin Tests ; Protozoan **Proteins** --analysis--AN; Sarcocystosis--immunology--IM; Trypsin--pharmacology--PD
CAS Registry No.: 0 (Antibodies, Protozoan); 0 (Membrane Proteins); 0 (Protozoan Proteins)
Enzyme No.: EC 3.4.21.4 (Trypsin)
Record Date Created: 19980514
Record Date Completed: 19980514

5/9/12
DIALOG(R)File 155:MEDLINE(R)

11952574 PMID: 9234899

Micropreparative high resolution purification of proteins by a combination of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, isoelectric focusing, and membrane blotting.

Liang F T; Granstrom D E; Timoney J F; Shi Y F
Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington 40546, USA.
Analytical biochemistry (UNITED STATES) Jul 15 1997, 250 (1) p61-5, ISSN 0003-2697 Journal Code: 0370535
Publishing Model Print

5/9/10

DIALOG(R)File 155:MEDLINE(R)

12575125 PMID: 9920311

Meningoencephalitis due to a Sarcocystis neurona **-like protozoan in Pacific harbor seals (Phoca vitulina richardsi).**

Lapointe J M; Duignan P J; Marsh A E; Gulland F M; Barr B C; Naydan D K; King D P; Farman C A; Huntingdon K A; Lowenstine L J

Departement de Pathologie, Faculte de Medecine Veterinaire, Universite de Montreal, St-Hyacinthe, Quebec, Canada.

Journal of parasitology (UNITED STATES) Dec 1998, 84 (6) p1184-9, ISSN 0022-3395 Journal Code: 7803124

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Seven Pacific harbor seals with meningoencephalitis associated with **Sarcocystis neurona** -like protozoa are described. Six of the 7 seals were free-ranging and were found stranded over an 80-km stretch of central California coastline; the other was captive. All had marked to severe nonsuppurative meningoencephalitis, most severe in the cerebellar cortex. Immunohistochemistry for S. **neurona antigens** was positive on brain tissue in all cases, revealing numerous merozoites as well as developing and mature schizonts, including rosette forms. Electron microscopy performed on 3 animals revealed merozoites and schizonts consistent with **Sarcocystis** sp., with the absence of rhoptries in merozoites, lack of a parasitophorous vacuole around schizonts, and division by endopolygeny. Serology using western blotting revealed the presence of anti-S. **neurona** immunoglobulins in the sera of 4 of 5 seals tested. Four animals also had a concurrent mild to moderate nonsuppurative myocarditis; in 1 seal, rare sarcocysts of undetermined species were present within cardiomyocytes.

Tags: Female; Male; Pregnancy

Descriptors: *Meningoencephalitis--veterinary--VE; * **Sarcocystis** --isolation and purification--IP; *Sarcocystosis--veterinary--VE; *Seals, Earless--parasitology--PS; Animals; Antibodies, Protozoan--blood--BL; Cerebellar Cortex--parasitology--PS; Cerebellar Cortex--pathology--PA; Immunohistochemistry; Meningoencephalitis--parasitology--PS; Meningoencephalitis--pathology--PA; Microscopy, Electron--veterinary--VE; Placenta --pathology--PA; Pregnancy; Pregnancy Complications, Parasitic --parasitology--PS; Pregnancy Complications, Parasitic--pathology--PA; Pregnancy Complications, Parasitic--veterinary--VE; **Sarcocystis** --immunology--IM; **Sarcocystis** --ultrastructure--UL; Sarcocystosis --parasitology--PS; Sarcocystosis--pathology--PA

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 19990203

Record Date Completed: 19990203

5/9/11

DIALOG(R)File 155:MEDLINE(R)

12263217 PMID: 9573058

Evidence that surface proteins **Sn14 and Sn16 of** Sarcocystis neurona **merozoites are involved in infection and** immunity .

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    $9.75  Estimated cost this search
   $11.28  Estimated total session cost   1.961 DialUnits


SYSTEM:OS  - DIALOG OneSearch
You have  26 files in your file list.
(To see file names, coverage dates, and copyright notices, enter SHOW FILES.)

      Set  Items  Description
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Added File(s): 5, 34, 35, 48, 65, 71, 73, 91, 94, 98, 135, 144,
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               467
Previous sets have been retained; enter DISPLAY SETS to view them.
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>>>One or more prefixes are unsupported
>>>  or undefined in one or more files.
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              90  SARCOSPORIDIA
             104  SARCOCYSTIDAE
               0
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            1448  S1
          809310  NEURONA?
      S2     192  S1 AND NEURONA?
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>>>  or undefined in one or more files.
>>>Year ranges not supported in one or more files
Processing
Processed  20 of  26 files ...
Completed processing all files
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        22555459  PY=2001 : PY=2005
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             192  S2
             120  S3
      S4      72  S2 NOT S3
Processing
Processed  10 of  26 files ...
Processing
Processed  20 of  26 files ...
Completed processing all files
              72  S4
         3060101  ANTIGEN?
        11312403  PROTEIN?
          847757  VACCIN?
         1235522  IMMUNI?
      S5      15  S4 AND (ANTIGEN? OR PROTEIN? OR VACCIN? OR IMMUNI?)
? ds

Set     Items   Description
S1       1448   R1-R5
S2        192   S1 AND NEURONA?
S3        120   S2/2001:2005
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S4          72   S2 NOT S3
S5          15   S4 AND (ANTIGEN? OR PROTEIN? OR VACCIN? OR IMMUNI?)
? ds

Set     Items   Description
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S3        120   S2/2001:2005
S4         72   S2 NOT S3
S5         15   S4 AND (ANTIGEN? OR PROTEIN? OR VACCIN? OR IMMUNI?)
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5/6/1 (Item 1 from file: 155)
13541431 PMID: 10511862
Serologic prevalence of Sarcocystis neurona , **Toxoplasma gondii, and Neospora caninum in horses in Brazil.**
Oct 1 1999

5/6/2 (Item 2 from file: 155)
13518527 PMID: 10489203
Prevalence of antibodies to Sarcocystis neurona , **Toxoplasma gondii and Neospora caninum in horses from Argentina.**
Sep 15 1999

5/6/3 (Item 3 from file: 155)
13165399 PMID: 11155929
The South American opossum, Didelphis marsupialis, from Brazil as another **definitive host for** Sarcocystis **speeri Dubey and Lindsay, 1999.**
Dec 2000

5/6/4 (Item 4 from file: 155)
13151015 PMID: 11128499
Immunohistochemical confirmation of Sarcocystis neurona **infections in raccoons, mink, cat, skunk, and pony.**
Oct 2000

5/6/5 (Item 5 from file: 155)
12999317 PMID: 10958438
In vitro cultivation of schizonts of Sarcocystis **speeri Dubey and Lindsay, 1999.**
Aug 2000

5/6/6 (Item 6 from file: 155)
12992935 PMID: 10946139
Inoculation of Sarcocystis neurona **merozoites into the central nervous system of horses.**
Sep 20 2000

5/6/7 (Item 7 from file: 155)
12758328 PMID: 10690772
Improvement of western blot test specificity for detecting equine serum antibodies to Sarcocystis neurona .

Jan 2000

5/6/8 (Item 8 from file: 155)
12685232 PMID: 10608440
Prevalence of antibodies to Neospora sp. in horses from Alabama and characterisation of an isolate recovered from a naturally infected horse [corrected]
Oct 1999

5/6/9 (Item 9 from file: 155)
12656705 PMID: 10577729
Sarcocystis **speeri N. sp. (Protozoa:** Sarcocystidae **) from the opossum (Didelphis virginiana).**
Oct 1999

5/6/10 (Item 10 from file: 155)
12575125 PMID: 9920311
Meningoencephalitis due to a Sarcocystis neurona **-like protozoan in Pacific harbor seals (Phoca vitulina richardsi).**
Dec 1998

5/6/11 (Item 11 from file: 155)
12263217 PMID: 9573058
Evidence that surface proteins **Sn14 and Sn16 of** Sarcocystis neurona **merozoites are involved in infection and** immunity **.**
May 1998

5/6/12 (Item 12 from file: 155)
11952574 PMID: 9234899
Micropreparative high resolution purification of proteins **by a combination of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, isoelectric focusing, and membrane blotting.**
Jul 15 1997

5/6/13 (Item 13 from file: 155)
11633290 PMID: 8944807
Neosporosis as a cause of equine protozoal myeloencephalitis.
Dec 1 1996

5/6/14 (Item 14 from file: 155)
10136275 PMID: 8466988
Equine protozoal myeloencephalitis: antigen **analysis of cultured** Sarcocystis neurona **merozoites.**
Jan 1993

5/6/15 (Item 15 from file: 155)
09863907 PMID: 1644935
A five year (1985-1989) retrospective study of equine neurological diseases with special reference to rabies.

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May 1992
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Dialog level 05.05.00D

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  File  91:MANTIS(TM) 1880-2005/May
         2005 (c) Action Potential
  File  94:JICST-EPlus 1985-2005/Apr W4
         (c)2005 Japan Science and Tech Corp(JST)
  File  98:General Sci Abs/Full-Text 1984-2004/Dec
         (c) 2005 The HW Wilson Co.
  File 135:NewsRx Weekly Reports 1995-2005/Jun W1
         (c) 2005 NewsRx
*File 135: New newsletters are now added. See Help News135 for the
complete list of newsletters.
  File 144:Pascal 1973-2005/Jun W1
         (c) 2005 INIST/CNRS
  File 149:TGG Health&Wellness DB(SM) 1976-2005/Jun W1
         (c) 2005 The Gale Group
  File 156:ToxFile 1965-2005/Jun W2
         (c) format only 2005 The Dialog Corporation
*File 156: ToxFile has been reloaded with the 2005 MeSH.
Please see HELP NEWS 156 for details.
  File 159:Cancerlit 1975-2002/Oct
         (c) format only 2002 Dialog Corporation
*File 159: Cancerlit is no longer updating.
Please see HELP NEWS159.
  File 162:Global Health 1983-2005/May
         (c) 2005 CAB International
  File 164:Allied & Complementary Medicine 1984-2005/Jun
         (c) 2005 BLHCIS
  File 172:EMBASE Alert 2005/Jun 08
         (c) 2005 Elsevier Science B.V.
  File 266:FEDRIP 2005/Jun
         Comp & dist by NTIS, Intl Copyright All Rights Res
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asymptomatic horses routinely submitted for equine infectious anaemia virus infection testing. We also subjected a 13-year-old horse with CNS disease to necropsy examination for isolation and in vitro cultivation of protozoal organisms. In antemortem tests, this horse was positive for antibodies to Neospora sp. in the IFAT and western immunoblot. Results of the prevalence survey indicated that IgG antibodies to Neospora were present in 62 (11.5%) of the 536 serum samples. Endpoint titres for the positive samples were 1:50 (35/6.5%), 1:100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). Tachyzoites were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the horse with CNS disease. Tachyzoites reacted with known N. caninum-positive serum from horses, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or equine anti- **Sarcocystis neurona** serum. Ultrastructural features of tachyzoites and results of comparison of tachyzoite immunodominant **proteins** revealed that they were identical to those of N. hughesi, a species described recently from a naturally infected horse. The isolate recovered from the naturally infected horse in the present study (designated NA1) is thought to be an isolate of N. hughesi, although confirmation of this awaits additional molecular characterisation. These results provide some additional evidence that N. hughesi is a valid species and that Neospora infections in horses may occur in widely separated geographic regions of the United States.

Tags: Female; Research Support, Non-U.S. Gov't

Descriptors: *Antibodies, Protozoan--blood--BL; *Coccidiosis--veterinary --VE; *Horse Diseases--epidemiology--EP; *Neospora--immunology--IM; *Neospora--isolation and purification--IP; Animals; Antibodies, Protozoan --immunology--IM; Cattle; Coccidiosis--epidemiology--EP; Coccidiosis --parasitology--PS; Dogs; Fluorescent Antibody Technique, Indirect; Horse Diseases--parasitology--PS; Horses; Mice; Myelitis--parasitology--PS; Myelitis--veterinary--VE; Neospora--ultrastructure--UL; Prevalence; Spinal Cord--parasitology--PS

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20000120

Record Date Completed: 20000120

5/9/12 (Item 12 from file: 155)

11952574 PMID: 9234899

Micropreparative high resolution purification of proteins **by a combination of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, isoelectric focusing, and membrane blotting.**

Liang F T; Granstrom D E; Timoney J F; Shi Y F

Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington 40546, USA.

Analytical biochemistry (UNITED STATES) Jul 15 1997, 250 (1) p61-5, ISSN 0003-2697 Journal Code: 0370535

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

We report a simple, economical, and efficient protocol for **protein** purification from cells. First, **proteins** of cell lysates were separated by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted to **protein** -blotting membrane. The blots

purification--IP; Animals; Antibodies, Protozoan--cerebrospinal fluid--CF; Antibodies, Protozoan--immunology--IM; **Antigens** , Protozoan--analysis--AN ; Coccidiosis--parasitology--PS; Encephalomyelitis--parasitology--PS; Horses; Immunohistochemistry; Neospora--immunology--IM; Neospora --ultrastructure--UL; Spinal Cord--parasitology--PS; Spinal Cord --ultrastructure--UL

CAS Registry No.: 0 (Antibodies, Protozoan); 0 (Antigens, Protozoan)

Record Date Created: 19970130

Record Date Completed: 19970130

5/9/14 (Item 14 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

10136275 PMID: 8466988

Equine protozoal myeloencephalitis: antigen **analysis of cultured** Sarcocystis neurona **merozoites.**

Granstrom D E; Dubey J P; Davis S W; Fayer R; Fox J C; Poonacha K B; Giles R C; Comer P F

Department of Veterinary Science, University of Kentucky, Lexington 40546-0099.

Journal of veterinary diagnostic investigation - official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc (UNITED STATES) Jan 1993, 5 (1) p88-90, ISSN 1040-6387

Journal Code: 9011490

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Antigens of cultured **Sarcocystis neurona** merozoites were examined using immunoblot analysis. Blotted **proteins** were probed with S. cruzi, S. muris, and S. **neurona** antisera produced in rabbits, S. fayeri (pre- and post-infection) and S. **neurona** (pre- and post-inoculation) sera produced in horses, immune sera from 7 histologically confirmed cases of equine protozoal myeloencephalitis (EPM), and pre-suckle serum from a newborn foal. Eight **proteins** , 70, 24, 23.5, 22.5, 13, 11, 10.5, and 10 Kd, were detected only by S. **neurona** antiserum and/or immune serum from EPM-affected horses. Equine sera were titered by the indirect immunofluorescent antibody (IFA) method using air-dried, cultured S. **neurona** merozoites. Anti- **Sarcocystis** IFA titers were found in horses with or without EPM. Serum titers did not correspond to the number of specific bands recognized on immunoblots.

Tags: Research Support, Non-U.S. Gov't

Descriptors: ***Antigen** s, Protozoan--analysis--AN; *Horse Diseases; * **Sarcocystis** --immunology--IM; *Sarcocystosis--veterinary--VE; Animals; **Antigens** , Protozoan--isolation and purification--IP; Cattle; Cells, Cultured; Electrophoresis, Polyacrylamide Gel; Horses; Immunoblotting; Molecular Weight; **Sarcocystis** --isolation and purification--IP

CAS Registry No.: 0 (Antigens, Protozoan)

Record Date Created: 19930510

Record Date Completed: 19930510

5/9/15 (Item 15 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09863907 PMID: 1644935

A five year (1985-1989) retrospective study of equine neurological diseases with special reference to rabies.

Hamir A N; Moser G; Rupprecht C E

Laboratory of Large Animal Pathology, University of Pennsylvania, New Bolton Center, Kennett Square 19348.

Journal of comparative pathology (ENGLAND) May 1992, 106 (4) p411-21 , ISSN 0021-9975 Journal Code: 0102444

Contract/Grant No.: AI-09206-16; AI; NIAID

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

A retrospective study of horses necropsied between 1985 and 1989 at a diagnostic laboratory of a veterinary school in North America is documented. In this investigation over 20 per cent of the horses had clinical neurological signs. Equine protozoal myeloencephalitis (caused by **Sarcocystis neurona**) and cervical stenotic myelopathy (wobbler syndrome) were the most common of these disorders. The veterinary school is located in the midst of a raccoon rabies enzootic area. However, only four cases of equine rabies were diagnosed during the 5-year study. The gross microscopical and immunohistochemical findings from these rabies-positive horses are documented. Immunoperoxidase tests for detection of rabies **antigen** in another 35 horses with non-specific encephalitis/encephalopathy did not reveal any positive cases. Based on this investigation, it appears that immunoperoxidase is a valid method for diagnosis of rabies when fresh tissues are not available for the fluorescent antibody test. It is also concluded that no cases of equine rabies were overlooked by the diagnostic laboratory during the period under investigation.

Tags: Female; Male; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Descriptors: *Horse Diseases--pathology--PA; *Nervous System Diseases --veterinary--VE; *Rabies--veterinary--VE; Animals; Brain--microbiology--MI ; Brain--pathology--PA; Diagnosis, Differential; Horse Diseases --epidemiology--EP; Horses; Immunoenzyme Techniques; Nervous System Diseases--complications--CO; Nervous System Diseases--epidemiology--EP; Nervous System Diseases--pathology--PA; Pennsylvania--epidemiology--EP; Rabies--complications--CO; Rabies--epidemiology--EP; Rabies--pathology --PA; Retrospective Studies; Spinal Cord--microbiology--MI; Spinal Cord --pathology--PA

Record Date Created: 19920910

Record Date Completed: 19920910

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File 34:SciSearch(R) Cited Ref Sci 1990-2005/Jun W1
(c) 2005 Inst for Sci Info
File 35:Dissertation Abs Online 1861-2005/May
(c) 2005 ProQuest Info&Learning
File 48:SPORTDiscus 1962-2005/Nov
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(c) 2005 BLDSC all rts. reserv.
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2005 (c) Action Potential
File 94:JICST-EPlus 1985-2005/Apr W4
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File 135:NewsRx Weekly Reports 1995-2005/Jun W1
(c) 2005 NewsRx
*File 135: New newsletters are now added. See Help News135 for the complete list of newsletters.
File 144:Pascal 1973-2005/Jun W1
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File 149:TGG Health&Wellness DB(SM) 1976-2005/Jun W1
(c) 2005 The Gale Group
File 156:ToxFile 1965-2005/Jun W2
(c) format only 2005 The Dialog Corporation
*File 156: ToxFile has been reloaded with the 2005 MeSH.
Please see HELP NEWS 156 for details.
File 159:Cancerlit 1975-2002/Oct
(c) format only 2002 Dialog Corporation
*File 159: Cancerlit is no longer updating.
Please see HELP NEWS159.
File 162:Global Health 1983-2005/May
(c) 2005 CAB International
File 164:Allied & Complementary Medicine 1984-2005/Jun
(c) 2005 BLHCIS
File 172:EMBASE Alert 2005/Jun 08
(c) 2005 Elsevier Science B.V.
File 266:FEDRIP 2005/Jun
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File 369:New Scientist 1994-2005/Apr W4
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File 370:Science 1996-1999/Jul W3
(c) 1999 AAAS
*File 370: This file is closed (no updates). Use File 47 for more current information.
File 399:CA SEARCH(R) 1967-2005/UD=14225
(c) 2005 American Chemical Society
*File 399: Use is subject to the terms of your user/customer agreement.
Alert feature enhanced for multiple files, etc. See HELP ALERT.
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File 444:New England Journal of Med. 1985-2005/May W5

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*File 467: F467 no longer updates; see Help News467.                    7.

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? sf allscience
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          847757  VACCIN?
      S6     642  NEURONA? (100N) VACCIN?
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Cultured; Electrophoresis, Polyacrylamide Gel; Horses; Immunoblotting; Molecular Weight; **Sarcocystis** --isolation and purification--IP
CAS Registry No.: 0 (Antigens, Protozoan)
Record Date Created: 19930510
Record Date Completed: 19930510

5/9/15
DIALOG(R)File 155:MEDLINE(R)

09863907 PMID: 1644935
A five year (1985-1989) retrospective study of equine neurological diseases with special reference to rabies.
Hamir A N; Moser G; Rupprecht C E
Laboratory of Large Animal Pathology, University of Pennsylvania, New Bolton Center, Kennett Square 19348.
Journal of comparative pathology (ENGLAND) May 1992, 106 (4) p411-21 , ISSN 0021-9975 Journal Code: 0102444
Contract/Grant No.: AI-09206-16; AI; NIAID
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
A retrospective study of horses necropsied between 1985 and 1989 at a diagnostic laboratory of a veterinary school in North America is documented. In this investigation over 20 per cent of the horses had clinical neurological signs. Equine protozoal myeloencephalitis (caused by **Sarcocystis neurona**) and cervical stenotic myelopathy (wobbler syndrome) were the most common of these disorders. The veterinary school is located in the midst of a raccoon rabies enzootic area. However, only four cases of equine rabies were diagnosed during the 5-year study. The gross microscopical and immunohistochemical findings from these rabies-positive horses are documented. Immunoperoxidase tests for detection of rabies **antigen** in another 35 horses with non-specific encephalitis/encephalopathy did not reveal any positive cases. Based on this investigation, it appears that immunoperoxidase is a valid method for diagnosis of rabies when fresh tissues are not available for the fluorescent antibody test. It is also concluded that no cases of equine rabies were overlooked by the diagnostic laboratory during the period under investigation.
Tags: Female; Male; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.
Descriptors: *Horse Diseases--pathology--PA; *Nervous System Diseases --veterinary--VE; *Rabies--veterinary--VE; Animals; Brain--microbiology--MI ; Brain--pathology--PA; Diagnosis, Differential; Horse Diseases --epidemiology--EP; Horses; Immunoenzyme Techniques; Nervous System Diseases--complications--CO; Nervous System Diseases--epidemiology--EP; Nervous System Diseases--pathology--PA; Pennsylvania--epidemiology--EP; Rabies--complications--CO; Rabies--epidemiology--EP; Rabies--pathology --PA; Retrospective Studies; Spinal Cord--microbiology--MI; Spinal Cord --pathology--PA
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     $6.34  Estimated total session cost   0.786 DialUnits

File 411:DIALINDEX(R)

DIALINDEX(R)
   (c) 2005 The Dialog Corporation

*** DIALINDEX search results display in an abbreviated ***
*** format unless you enter the SET DETAIL ON command. ***
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   (To see banners, use SHOW FILES command)
? s neurona? (100n) vaccin?

Your SELECT statement is:
   s neurona? (100n) vaccin?

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N74            1   635: Business Dateline(R)_1985-2005/Jun 11
N75            1   750: Emerging Mkts & Middle East News_1995-2005/Jun 13
N76            1   764: BCC Market Research_1989-2005/Jun
N77            1   765: Frost & Sullivan_1992-1999/Apr
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     $7.84  Estimated cost this search
    $14.18  Estimated total session cost   3.344 DialUnits


SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R) 1951-2005/Jun W2
        (c) format only 2005 The Dialog Corp.
  File 654:US Pat.Full. 1976-2005/Jun 09
        (c) Format only 2005 The Dialog Corp.
  File 349:PCT FULLTEXT 1979-2005/UB=20050609,UT=20050602
        (c) 2005 WIPO/Univentio
  File   5:Biosis Previews(R) 1969-2005/Jun W1
        (c) 2005 BIOSIS
  File  73:EMBASE 1974-2005/Jun W1
        (c) 2005 Elsevier Science B.V.
  File 357:Derwent Biotech Res. _1982-2005/Jun W1
        (c) 2005 Thomson Derwent & ISI
  File 370:Science 1996-1999/Jul W3
        (c) 1999 AAAS
```

***File 370: This file is closed (no updates). Use File 47 for more current information.**
File 203:AGRIS 1974-2005/Feb
Dist by NAL, Intl Copr. All rights reserved
File 185:Zoological Record Online(R) 1978-2005/Jun
(c) 2005 BIOSIS
File 143:Biol. & Agric. Index 1983-2005/May
(c) 2005 The HW Wilson Co
File 160:Gale Group PROMT(R) 1972-1989
(c) 1999 The Gale Group
File 35:Dissertation Abs Online 1861-2005/May
(c) 2005 ProQuest Info&Learning
File 94:JICST-EPlus 1985-2005/Apr W4
(c)2005 Japan Science and Tech Corp(JST)
File 10:AGRICOLA 70-2005/Jun
(c) format only 2005 The Dialog Corporation
File 444:New England Journal of Med. 1985-2005/May W5
(c) 2005 Mass. Med. Soc.
File 129:PHIND(Archival) 1980-2005/Jun W1
(c) 2005 T&F Informa UK Ltd
***File 129: Effective 1 May 2005, ERA-News will change to EURALex. See HELP NEWS 130 for details.**
File 348:EUROPEAN PATENTS 1978-2005/Jun W02
(c) 2005 European Patent Office
File 16:Gale Group PROMT(R) 1990-2005/Jun 13
(c) 2005 The Gale Group
File 399:CA SEARCH(R) 1967-2005/UD=14225
(c) 2005 American Chemical Society
***File 399: Use is subject to the terms of your user/customer agreement.**
Alert feature enhanced for multiple files, etc. See HELP ALERT.
File 50:CAB Abstracts 1972-2005/May
(c) 2005 CAB International

```
      Set  Items  Description
      ---  -----  -----------
Executing TD66926426
>>>SET HILIGHT: use ON, OFF, or 1-5 characters
          480869  NEURONA?
          760172  VACCIN?
      S1    1096  NEURONA? (100N) VACCIN?
? s s1 and (epm or equine? or sarcocysti?)
            1096  S1
            9928  EPM
          114608  EQUINE?
           23686  SARCOCYSTI?
      S2     292  S1 AND (EPM OR EQUINE? OR SARCOCYSTI?)
? s s2 and sarcocy?
             292  S2
           24094  SARCOCY?
      S3      84  S2 AND SARCOCY?
? s s2 and epm
             292  S2
            9928  EPM
      S4      46  S2 AND EPM
? s s3 or s4
              84  S3
              46  S4
      S5      88  S3 OR S4
? s s5 and (antigen? or protein?)
```

```
Processing
>>>File 349 processing for PROTEIN? stopped at PROTEINENG5
Processing
Processed  10 of  20 files ...
>>>File 348 processing for PROTEIN? stopped at PROTEINSEQUENZIERUNG
Processing
Completed processing all files
              88  S5
         2231646  ANTIGEN?
         8637004  PROTEIN?
      S6      55  S5 AND (ANTIGEN? OR PROTEIN?)
? s s5 and cell?
>>>File 654 processing for CELL? stopped at CELLSTAB
Processing
>>>File 349 processing for CELL? stopped at CELLLINE8ML4
Processing
Processing
Processed  10 of  20 files ...
>>>File 348 processing for CELL? stopped at CELLULOSECARBAMATLOSUNG
Processing
Processed  20 of  20 files ...
Completed processing all files
              88  S5
        14712333  CELL?
      S7      51  S5 AND CELL?
? s s5 and (merozoi? or tachyzo?)
              88  S5
           15172  MEROZOI?
            6357  TACHYZO?
      S8      25  S5 AND (MEROZOI? OR TACHYZO?)
?
? ds

Set     Items   Description
S1       1096   NEURONA? (100N) VACCIN?
S2        292   S1 AND (EPM OR EQUINE? OR SARCOCYSTI?)
S3         84   S2 AND SARCOCY?
S4         46   S2 AND EPM
S5         88   S3 OR S4
S6         55   S5 AND (ANTIGEN? OR PROTEIN?)
S7         51   S5 AND CELL?
S8         25   S5 AND (MEROZOI? OR TACHYZO?)
? s s6 and s7 and s8
              55  S6
              51  S7
              25  S8
      S9      19  S6 AND S7 AND S8
? t s9/free/all
>>>"FREE" is not a valid format name in file(s): 348-349, 399, 654

 9/8/1     (Item 1 from file: 155)
DIALOG(R)File 155:(c) format only 2005 The Dialog Corp. All rts. reserv.

12263217   PMID: 9573058
   Evidence that surface proteins Sn14 and Sn16 of Sarcocystis neurona
 merozoites are involved in infection and immunity.
May 1998
  Tags: Research Support, Non-U.S. Gov't
  Descriptors:  *Encephalitis--veterinary--VE;  *Horse Diseases--immunology
```

--IM; *Protozoan **Proteins** --immunology--IM; * **Sarcocystis** --immunology--IM ; * **Sarcocystosis** --veterinary--VE; Animals; Antibodies, Protozoan --immunology--IM; Encephalitis--immunology--IM; Horses; Immunoblotting; Membrane **Proteins** --immunology--IM; Neutralization Tests; Precipitin Tests ; Protozoan **Proteins** --analysis--AN; **Sarcocystosis** --immunology--IM; Trypsin--pharmacology--PD
CAS Registry No.: 0 (Antibodies, Protozoan); 0 (Membrane Proteins); 0 (Protozoan Proteins)
Enzyme No.: EC 3.4.21.4 (Trypsin)

9/8/16 (Item 1 from file: 5)
0011463458 BIOSIS NO.: 199800257705
Evidence that surface proteins **Sn14 and Sn16 of** Sarcocystis **neurona** merozoites **are involved in infection and immunity**
1998

9/8/17 (Item 1 from file: 357)
0334536 DBR Accession No.: 2004-06828
New nucleic acid capable of encoding antigenic proteins **derived from** Sarcocystis **neurona, useful in detecting, preventing and treating** Sarcocystis **neurona infections, e.g.** equine **protozoal myeloencephalitis - recombinant** protein **production for use** in disease **therapy and gene therapy** 2004

9/8/18 (Item 2 from file: 357)
0302369 DBR Accession No.: 2003-04154
Composition for detecting presence of Sarcocystis neurona **in a biological samples, comprises a SnSAG-1 marker e.g. a SnSAG-1 nucleic acid, SnSAG-1** protein **or an antibody that specifically binds to SnSAG-1** protein **- vector-mediated gene transfer and expression in host** cell **for recombinant** vaccine **and diagnosis** 2002

9/8/19 (Item 3 from file: 357)
0280356 DBR Accession No.: 2002-04497
Vaccine useful for preventing or ameliorating equine **protozoal myeloencephalitis disease, comprises inactivated** Sarcocystis **neuroma** cells **and/or Neospora hughesi** cells , antigens , **DNA derived from the** cells **or their mixtures - horse protozoon myeloencephalitis disease therapy suing a recombinant vaccine or a nucleic acid vaccine**
2001
? ds

Set	Items	Description
S1	1096	NEURONA? (100N) VACCIN?
S2	292	S1 AND (EPM OR EQUINE? OR SARCOCYSTI?)
S3	84	S2 AND SARCOCY?
S4	46	S2 AND EPM
S5	88	S3 OR S4
S6	55	S5 AND (ANTIGEN? OR PROTEIN?)
S7	51	S5 AND CELL?
S8	25	S5 AND (MEROZOI? OR TACHYZO?)
S9	19	S6 AND S7 AND S8

? s s6 or s7 or s8

```
              55  S6
              51  S7
              25  S8
     S10      67  S6 OR S7 OR S8
? t s10/6/all
```

10/6/1 (Item 1 from file: 155)
16561874 PMID: 15517384
Sarcocystis **neurona major surface** antigen **gene 1 (SAG1) shows evidence of having evolved under positive selection pressure.**
Dec 2004

10/6/2 (Item 2 from file: 155)
15346283 PMID: 15150728
Evaluation of immune responses in horses immunized using a killed Sarcocystis neurona vaccine .
Spring 2004

10/6/3 (Item 3 from file: 155)
14257575 PMID: 12062508
Seroprevalence of Neospora, Toxoplasma gondii and Sarcocystis **neurona antibodies in horses from Jeju island, South Korea.**
Jun 26 2002

10/6/4 (Item 4 from file: 155)
12992935 PMID: 10946139
Inoculation of Sarcocystis **neurona** merozoites **into the central nervous system of horses.**
Sep 20 2000

10/6/5 (Item 5 from file: 155)
12263217 PMID: 9573058
Evidence that surface proteins **Sn14 and Sn16 of** Sarcocystis **neurona** merozoites **are involved in infection and immunity.**
May 1998

10/6/6 (Item 1 from file: 654)
5993734
Derwent Accession: 2005-161937
UTILITY
Detection of sarcocystis **neurona**

Fulltext Word Count: 19644
Number of Claims: 13
Exemplary or Independent Claim Number(s): 1,6,12

10/6/7 (Item 2 from file: 654)
5845932
Derwent Accession: 2002-712484
Utility
Detection of sarcocystis **neurona**

Fulltext Word Count: 19327
Number of Claims: 11
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 6
Number of non-US cited patent references: 2
Number of non-patent cited references: 5

10/6/8 (Item 3 from file: 654)
5767158 **IMAGE Available
Derwent Accession: 2004-200933
Utility
Animal model for infection by an apicomplexan parasite

Fulltext Word Count: 12713
Number of Claims: 12
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 7
Number of Figures: 7
Number of US cited patent references: 1
Number of non-patent cited references: 33

10/6/9 (Item 4 from file: 654)
0005764804 **IMAGE Available
Derwent Accession: 2004-614854
Nucleic acids encoding Sarcocystis **neurona** antigen **and uses thereof**

Fulltext Word Count: 18910
Number of Claims: 20
Exemplary or Independent Claim Number(s): 1,7,11,13,17,18
Number of Drawing Sheets: 5
Number of Figures: 5

10/6/10 (Item 5 from file: 654)
5623371 **IMAGE Available
Derwent Accession: 2002-339775
Utility
C/ **EIAV p26 deletion vaccine and diagnostic**

Fulltext Word Count: 12053
Number of Claims: 12
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 14
Number of Figures: 22
Number of non-US cited patent references: 1

10/6/11 (Item 6 from file: 654)
0005447919 **IMAGE Available
Derwent Accession: 2004-200933
Animal model for infection by an apicomplexan parasite

Fulltext Word Count: 15351
Number of Claims: 30
Exemplary or Independent Claim Number(s): 1,7,12,17,19,20,26,28,29,29,30
Number of Drawing Sheets: 7
Number of Figures: 7

10/6/12 (Item 7 from file: 654)
0005320850 **IMAGE Available
Derwent Accession: 2003-586973
Composition & corresponding method of using spores of bacillus subtilis to stimulate and/or enhance immune response in mammals

Fulltext Word Count: 18052
Number of Claims: 33
Exemplary or Independent Claim Number(s): 1,14,19,24,28,30,32,33
Number of Drawing Sheets: 20
Number of Figures: 20

10/6/13 (Item 8 from file: 654)
0005252167
Derwent Accession: 2003-708725
Method and composition for excystation of sporocysts

Fulltext Word Count: 5804
Number of Claims: 45
Exemplary or Independent Claim Number(s): 1,21,33,37

10/6/14 (Item 9 from file: 654)
0005174489 **IMAGE Available
Derwent Accession: 2002-339775
EIAV P26 deletion vaccine and diagnostic

Fulltext Word Count: 14549
Number of Claims: 25
Exemplary or Independent Claim Number(s): 1,2,17,22,23,25
Number of Drawing Sheets: 14
Number of Figures: 14

10/6/15 (Item 10 from file: 654)
0005169483 **IMAGE Available
Derwent Accession: 2002-339775
EIAV p26 deletion vaccine and diagnostic

Fulltext Word Count: 14499
Number of Claims: 25
Exemplary or Independent Claim Number(s): 1,2,17,22,23,25
Number of Drawing Sheets: 14
Number of Figures: 15

10/6/16 (Item 11 from file: 654)
0005100373 **IMAGE Available
Derwent Accession: 2002-339775
EIAV p26 deletion vaccine and diagnostic

Fulltext Word Count: 14500
Number of Claims: 25
Exemplary or Independent Claim Number(s): 1,2,17,22,23,25
Number of Drawing Sheets: 13
Number of Figures: 14

10/6/17 (Item 12 from file: 654)
0005061084
Derwent Accession: 2002-712484
Detection of sarcocystis **neurona**

Fulltext Word Count: 23294
Number of Claims: 56
Exemplary or Independent Claim Number(s): 1,24,50

10/6/18 (Item 13 from file: 654)
0004987264
Derwent Accession: 2002-049244
Equine **protozoal myeloencephalitis vaccine**

Fulltext Word Count: 6180
Number of Claims: 25
Exemplary or Independent Claim Number(s): 1,15,18,19,20,23

10/6/19 (Item 14 from file: 654)
4760127 **IMAGE Available
Derwent Accession: 2002-339775
Utility
C/ **EIAV p26 deletion vaccine and diagnostic**
; **IMMUNOGENIC COMPOSITION THAT PRODUCES PROTECTION FROM** EQUINE **INFECTIOUS ANEMIA DISEASE COMPRISING NON-REPLICATING** EQUINE **INFECTIOUS ANEMIA VIRUS THAT LACKS ABILITY TO EXPRESS CAPSID** ANTIGEN

Fulltext Word Count: 12019
Number of Claims: 11
Exemplary or Independent Claim Number(s): 1
Number of Drawing Sheets: 14
Number of Figures: 22
Number of US cited patent references: 2
Number of non-patent cited references: 37

10/6/20 (Item 15 from file: 654)
4745296
Derwent Accession: 1998-110331
Utility
C/ **Treatment of** equine **protozoal myeloencephalitis**
; CAUSED **BY INFECTION BY THE PROTOZOAN PARASITE** SARCOCYSTIS **NEURONA (RECENTLY REFERRED TO AS** SARCOCYSTIS **FALCATULA)**

Fulltext Word Count: 4189
Number of Claims: 15
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 22
Number of non-US cited patent references: 1
Number of non-patent cited references: 54

10/6/21 (Item 16 from file: 654)
4654665

Derwent Accession: 2002-424659
Utility
C/ **Dirofilaria and Brugia ankyrin** proteins **and uses thereof**
; TO PROTE **CT ANIMALS FROM DISEASES CAUSED BY PARASITIC HELMINTHS**

Fulltext Word Count: 29792
Number of Claims: 18
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 2
Number of non-US cited patent references: 1
Number of non-patent cited references: 15

10/6/22 (Item 17 from file: 654)
4631468
Derwent Accession: 2000-571969
Utility
C/ Antigen **test to detect** equine **protozoal myeloencephalitis in horse serum and cerebrospinal fluid**
; AN IMMU NOASSA **Y DETECTION METHOD AND KIT TO DIAGNOSE HORSES INFECTED WITH** SARCOCYSTIS **NEURONA USING POLYCLONAL OR MONOCLONAL ANTIBODIES AGAINST THE PROTOZOAN** ANTIGEN ; **DIAGNOSTIC KITS**

Fulltext Word Count: 14935
Number of Claims: 36
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 38
Number of non-patent cited references: 18

10/6/23 (Item 18 from file: 654)
4532862
Derwent Accession: 1998-110331
Utility
C/ **Treatment of** equine **protozoal myeloencephalitis**
; VETERI **NARY MEDICINE; SYNERGISTIC MIXTURE OF PYRIMETHAMINE, SULFONAMIDE AND TRIMETHOPRIM**

Fulltext Word Count: 4511
Number of Claims: 29
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 13
Number of non-US cited patent references: 1
Number of non-patent cited references: 11

10/6/24 (Item 19 from file: 654)
4321500
Derwent Accession: 2000-375493
Utility
C/ **Dirofilaria and brugia ankyrin** proteins , **nucleic acid molecules, and uses thereof**
; NUCLEIC **ACID SEQUENCE OF PARASITE POLYPEPTIDE; FOR DIAGNOSIS OF PARASITIC HELMINTH INFECTION; FOR THE DEVELOPMENT OF VACCINES TO PREVENT HELMINTH AND NEMATODE INFECTION**

Fulltext Word Count: 29314
Number of Claims: 19

Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 2
Number of non-US cited patent references: 1
Number of non-patent cited references: 16

10/6/25 (Item 20 from file: 654)
4187514
Utility
C/ **Dirofilaria and brugia ankyrin** proteins , **nucleic acid molecules, and uses thereof**
; PATENT N **OT GRANTED PER USPTO**

Fulltext Word Count: 23715
Number of Claims: 14
Exemplary or Independent Claim Number(s): 1
Number of non-patent cited references: 12

10/6/26 (Item 21 from file: 654)
4064257
Derwent Accession: 1998-609296
Utility
C/ **Treatment of** equine **protozoan myeloencephalitis using triazinediones**

Fulltext Word Count: 6526
Number of Claims: 27
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 8
Number of non-US cited patent references: 2
Number of non-patent cited references: 15

10/6/27 (Item 22 from file: 654)
4060786
Derwent Accession: 1998-593992
Utility
C/ **Dirofilaria and Brugia ankyrin** proteins , **nucleic acid molecules**, and **uses thereof**
; PARASITI **CIDES AGAINST HEARTWORM, ELEPHANTIASIS**

Fulltext Word Count: 23963
Number of Claims: 19
Exemplary or Independent Claim Number(s): 1
Number of non-patent cited references: 12

10/6/28 (Item 23 from file: 654)
4057127
Derwent Accession: 1998-593373
Utility
C/ **Dirofilaria and brugia ankyrin** proteins , **nucleic acid molecules, and uses thereof**
; ISOLATIO **N AND SEPARATION OF** PROTEINS **AND NUCLEIC ACID MOLECULES**

Fulltext Word Count: 23708
Number of Claims: 14
Exemplary or Independent Claim Number(s): 1

10/6/29 (Item 24 from file: 654)
3975964
Derwent Accession: 1998-110331
Utility
C/ **Treatment of** equine **protozoal myeloencephalitis**
; MIXTUR **E OF PYRIMETHAMINE AND A SULFONAMIDE COMPOUND**

Fulltext Word Count: 5012
Number of Claims: 48
Exemplary or Independent Claim Number(s): 1
Number of US cited patent references: 10
Number of non-patent cited references: 37

10/6/30 (Item 1 from file: 349)
01241209
VACCINE AND METHOD FOR TREATMENT OF NEURODEGENERATIVE DISEASES
VACCIN ET PROCEDE POUR TRAITER DES MALADIES NEURODEGENERATIVES
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 20121
Publication Year: 2005

10/6/31 (Item 2 from file: 349)
01106329
RIBOSWITCHES, METHODS FOR THEIR USE, AND COMPOSITIONS FOR USE WITH RIBOSWITCHES.
RIBOSWITCHS, PROCEDES D'UTILISATION ET COMPOSITIONS A UTILISER AVEC DES RIBOSWITCHS
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 67340
Publication Year: 2004

10/6/32 (Item 3 from file: 349)
01106055
LIVE ANTENUATED PARASITE VACCINE
VACCIN DE PARASITE VIVANT ATTENUE
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 12092
Publication Year: 2004

10/6/33 (Item 4 from file: 349)

01083800 **Image available**
NUCLEIC ACIDS ENCODING SARCOCYSTIC **NEURONA** ANTIGEN **AND USES THEREOF**
ACIDES NUCLEIQUES CODANT L' ANTIGENE **<I>SARCOSYSTIS NEURONA</I> ET LEURS UTILISATIONS**
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 17325
Publication Year: 2004

10/6/34 (Item 5 from file: 349)
01027073
BACILLUS SUBTILIS SPORES TO STIMULATE THE IMMUNE RESPONSES
APPLICATIONS DE SPORES
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 16542
Publication Year: 2003

10/6/35 (Item 6 from file: 349)
00886309 **Image available**
EIAV P26 DELETION VACCINE AND DIAGNOSTIC
VACCIN DE DELETION DE LA PROTEINE **P26 DU VIRUS DE L'ANEMIE INFECTIEUSE** EQUINE **(EIAV)**
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 11838
Publication Year: 2002

10/6/36 (Item 7 from file: 349)
00886308 **Image available**
EIAV CHIMERIC VACCINE AND DIAGNOSTIC
VACCIN CHIMERE CONTRE LE VIRUS DE L'ANEMIE INFECTIEUSE EQUINE **ET DIAGNOSTIC**
Publication Language: English
Filing Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 12669
Publication Year: 2002

10/6/37 (Item 8 from file: 349)
00849074
EQUINE **PROTOZOAL MYELOENCEPHALITIS VACCINE**
VACCIN CONTRE LA MYELOENCEPHALITE PROTOZOAIRE DU CHEVAL

Publication Language: English
Filing Language: English
Fulltext Availability:
  Detailed Description
  Claims
Fulltext Word Count: 5876
Publication Year: 2001

10/6/38 (Item 9 from file: 349)
00783804
VACCINE TO CONTROL EQUINE **PROTOZOAL MYELOENCEPHALITIS IN HORSES**
VACCIN DESTINE A COMBATTRE LA MYELOENCEPHALITE EQUINE **A PROTOZOAIRE**
Publication Language: English
Filing Language: English
Fulltext Availability:
  Detailed Description
  Claims
Fulltext Word Count: 13954
Publication Year: 2001

10/6/39 (Item 10 from file: 349)
00749379
FLEA HEAD, NERVE CORD, HINDGUT AND MALPIGHIAN TUBULE NUCLEIC ACID MOLECULES, PROTEINS AND USES THEREOF
MOLECULES D'ACIDES NUCLEIQUES ET PROTEINES ISSUES DE LA TETE, DE LA MOELLE EPINIERE, DE L'INTESTIN POSTERIEUR ET DU TUBE DE MALPIGHI DE PUCES ET UTILISATIONS CORRESPONDANTES
Publication Language: English
Filing Language: English
Fulltext Availability:
  Detailed Description
  Claims
Fulltext Word Count: 243455
Publication Year: 2000

10/6/40 (Item 11 from file: 349)
00736690
AN ANTIGEN **TEST TO DETECT** EQUINE **PROTOZOAL MYELOENCEPHALITIS IN HORSE SERUM AND CEREBROSPINAL FLUID**
TEST D' ANTIGENES **POUR LA DETECTION DE MYELOENCEPHALITE PROTOZOAIRE** EQUINE **DANS LE SERUM ET LE LIQUIDE CEPHALORACHIDIEN EQUINS**
Publication Language: English
Filing Language: English
Fulltext Availability:
  Detailed Description
  Claims
Fulltext Word Count: 15896
Publication Year: 2000

10/6/41 (Item 12 from file: 349)
00542799
TREATMENT OF EQUINE **PROTOZOAN MYELOENCEPHALITIS USING TRIAZINEDIONES**
TRAITEMENT DE LA MYELOENCEPHALITE PROTOZOAIRE EQUINE **A L'AIDE DE TRIAZINEDIONES**

Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 7129
Publication Year: 2000

10/6/42 (Item 13 from file: 349)
00457454
DIROFILARIA AND BRUGIA ANKYRIN PROTEINS, NUCLEIC ACID MOLECULES, AND USES THEREOF
PROTEINES ANKYRINES DE DIROFILARIA ET BRUGIA, MOLECULES D'ACIDE NUCLEIQUE ET LEURS UTILISATIONS
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 62164
Publication Year: 1998

10/6/43 (Item 14 from file: 349)
00411704
TREATMENT OF EQUINE **PROTOZOAL MYELOENCEPHALITIS**
TRAITEMENT DE LA MYELOENCEPHALITE PROTOZOAIRE EQUINE
Publication Language: English
Fulltext Availability:
Detailed Description
Claims
Fulltext Word Count: 4834
Publication Year: 1998

10/6/44 (Item 1 from file: 5)
0015191192 BIOSIS NO.: 200500097105
Sarcocystis **neurona major surface** antigen **gene 1 (SAG1) shows evidence of having evolved under positive selection pressure**
2004

10/6/45 (Item 2 from file: 5)
0015068179 BIOSIS NO.: 200400448968
Detection of sarcocystis **neurona**
2004

10/6/46 (Item 3 from file: 5)
0013812616 BIOSIS NO.: 200200406127
Seroprevalence of Neospora, Toxoplasma gondii and Sarcocystis **neurona antibodies in horses from Jeju island, South Korea**
2002

10/6/47 (Item 4 from file: 5)
0012751297 BIOSIS NO.: 200000469610
Inoculation of Sarcocystis **neurona** merozoites **into the central nervous system of horses**

2000

10/6/48 (Item 5 from file: 5)
0011463458 BIOSIS NO.: 199800257705
Evidence that surface proteins **Sn14 and Sn16 of** Sarcocystis **neurona** merozoites **are involved in infection and immunity**
1998

10/6/49 (Item 1 from file: 73)
12974201 EMBASE No: 2005033908
Sarcocystis **neurona major surface** antigen **gene 1 (SAG1) shows evidence of having evolved under positive selection pressure**
2004

10/6/50 (Item 2 from file: 73)
11633355 EMBASE No: 2002204930
Seroprevalence of Neospora, Toxoplasma gondii and Sarcocystis **neurona antibodies in horses from Jeju island, South Korea**
26 JUN 2002

10/6/51 (Item 3 from file: 73)
10814468 EMBASE No: 2000288590
Inoculation of Sarcocystis **neurona** merozoites **into the central nervous system of horses**
20 SEP 2000

10/6/52 (Item 1 from file: 357)
0349492 DBR Accession No.: 2004-21784
New composition comprising an isolated nucleic acid molecule encoding an antigenic protein **derived from** Sarcocystis neurona , **useful for developing** vaccines **for** equine **protozoal myeloencephalitis (EPM) or S.** neurona **- vector-mediated parasite** antigen **gene transfer and expression in host cell for recombinant** vaccine 2004

10/6/53 (Item 2 from file: 357)
0334536 DBR Accession No.: 2004-06828
New nucleic acid capable of encoding antigenic proteins **derived from** Sarcocystis **neurona, useful in detecting, preventing and treating** Sarcocystis **neurona infections, e.g.** equine **protozoal myeloencephalitis - recombinant** protein **production for use in disease therapy and gene therapy** 2004

10/6/54 (Item 3 from file: 357)
0333032 DBR Accession No.: 2004-05324
ApiEST-DB: analyzing clustered EST data of the apicomplexan parasites - protozoon clustered expressed sequence tag analysis and data mining for use in drug screening and diagnosis 2004

10/6/55 (Item 4 from file: 357)

0302369 DBR Accession No.: 2003-04154
Composition for detecting presence of Sarcocystis neurona **in a biological samples, comprises a SnSAG-1 marker e.g. a SnSAG-1 nucleic acid, SnSAG-1** protein **or an antibody that specifically binds to SnSAG-1** protein **- vector-mediated gene transfer and expression in host** cell **for recombinant** vaccine **and diagnosis** 2002

10/6/56 (Item 5 from file: 357)
0280356 DBR Accession No.: 2002-04497
Vaccine useful for preventing or ameliorating equine **protozoal myeloencephalitis disease, comprises inactivated** Sarcocystis **neuroma** cells **and/or Neospora hughesi** cells , antigens , **DNA derived from the** cells **or their mixtures - horse protozoon myeloencephalitis disease therapy suing a recombinant vaccine or a nucleic acid vaccine** 2001

10/6/57 (Item 6 from file: 357)
0267981 DBR Accession No.: 2001-07735
Vaccinating **equids against protozoal** Sarcocystis neurona **infections using** antigens **-** Sarcocystis neurona **nucleic acid** vaccine **and recombinant** vaccine 2001

10/6/58 (Item 1 from file: 185)
0002047753 BIOSIS No. 14103016971
Sarcocystis **neurona major surface** antigen **gene 1 (SAG1) shows evidence of having evolved under positive selection pressure.**
PUBLICATION YEAR: 2004

10/6/59 (Item 1 from file: 129)
00731140
Fort Dodge's EPM **(** equine **protozoal myeloencephalitis) vaccine licence renewed:**, November 02, 2001 (20011102)
STORY TYPE: B WORD COUNT: 174

10/6/60 (Item 1 from file: 399)
DIALOG(R)File 399:(c) 2005 American Chemical Society. All rts. reserv.

Protein and cDNA sequences of Sarcocystis neurona membrane-associated antigens and their uses in immunodiagnosis and immunotherapy of equine protozoal myeloencephalitis

10/6/61 (Item 2 from file: 399)
DIALOG(R)File 399:(c) 2005 American Chemical Society. All rts. reserv.

Sarcocystis neurona surface antigens, nucleic acids and antibodies for diagnosis, prevention and therapy

10/6/62 (Item 3 from file: 399)
DIALOG(R)File 399:(c) 2005 American Chemical Society. All rts. reserv.

Use of SAG-1 gene of Sarcocystis neurona for diagnostic tests and vaccines for equine protozoal myeloencephalitis

10/6/63 (Item 4 from file: 399)
DIALOG(R)File 399:(c) 2005 American Chemical Society. All rts. reserv.

Equine protozoal myeloencephalitis vaccine

10/6/64 (Item 5 from file: 399)
DIALOG(R)File 399:(c) 2005 American Chemical Society. All rts. reserv.

Vaccine to control equine protozoal myeloencephalitis in horses

10/6/65 (Item 1 from file: 50)
0008758161 CAB Accession Number: 20053009085
Sarcocystis **neurona major surface** antigen **gene 1 (SAG1) shows evidence of having evolved under positive selection pressure.**
Publication Year: 2004

10/6/66 (Item 2 from file: 50)
0008618298 CAB Accession Number: 20043070850
Evaluation of immune responses in horses immunized using a killed Sarcocystis neurona vaccine .
Publication Year: 2004

10/6/67 (Item 3 from file: 50)
0008241943 CAB Accession Number: 20023102736
Seroprevalence of Neospora , Toxoplasma gondii and Sarcocystis **neurona antibodies in horses from Jeju island, South Korea.**
Publication Year: 2002
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13jun05 15:20:20 User228206 Session D2455.13
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  File 357:Derwent Biotech Res. _1982-2005/Jun W1
        (c) 2005 Thomson Derwent & ISI
  File 370:Science 1996-1999/Jul W3
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*File 370: This file is closed (no updates). Use File 47 for more current
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        (c) 2005 ProQuest Info&Learning
  File  94:JICST-EPlus 1985-2005/Apr W4
        (c)2005 Japan Science and Tech Corp(JST)
  File  10:AGRICOLA 70-2005/Jun
        (c) format only 2005 The Dialog Corporation
  File 444:New England Journal of Med. 1985-2005/May W5
        (c) 2005 Mass. Med. Soc.
  File 129:PHIND(Archival) 1980-2005/Jun W1
        (c) 2005 T&F Informa UK Ltd
*File 129: Effective 1 May 2005, ERA-News will change to EURALex. See HELP
NEWS 130 for details.
  File 348:EUROPEAN PATENTS 1978-2005/Jun W02
        (c) 2005 European Patent Office
  File  16:Gale Group PROMT(R) 1990-2005/Jun 13
        (c) 2005 The Gale Group
  File 399:CA SEARCH(R) 1967-2005/UD=14225
        (c) 2005 American Chemical Society
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Alert feature enhanced for multiple files, etc. See HELP ALERT.
  File  50:CAB Abstracts 1972-2005/May
        (c) 2005 CAB International

      Set  Items  Description
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S3         84   S2 AND SARCOCY?
S4         46   S2 AND EPM
S5         88   S3 OR S4
S6         55   S5 AND (ANTIGEN? OR PROTEIN?)
S7         51   S5 AND CELL?
S8         25   S5 AND (MEROZOI? OR TACHYZO?)
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S9 19 S6 AND S7 AND S8
S10 67 S6 OR S7 OR S8
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10/9/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12992935 PMID: 10946139
Inoculation of Sarcocystis **neurona** merozoites **into the central nervous system of horses.**
Lindsay D S; Dykstra C C; Williams A; Spencer J A; Lenz S D; Palma K; Dubey J P; Blagburn B L
Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, 24061-0342, USA. lindsayd@vt.edu
Veterinary parasitology (NETHERLANDS) Sep 20 2000, 92 (2) p157-63, ISSN 0304-4017 Journal Code: 7602745
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Equine protozoal myeloencephalitis (**EPM**) is a neurologic syndrome in horses from the Americas and is usually caused by infection with the apicomplexan parasite, **Sarcocystis neurona** . A horse model of **EPM** is needed to test the efficacy of chemotherapeutic agents and potential **vaccines** . Five horses that were negative for antibodies to S. **neurona** in their serum and cerebrospinal fluid (CSF) were injected in the subarachnoid space with living **merozoites** of the SN2 isolate of S. **neurona** . None of the horses developed clinical disease or died over a 132-day observation period. All five horses developed antibodies to S. **neurona** in their CSF and serum 3-4 weeks after injection. Two of the horses were examined at necropsy and no parasite induced lesions were observed in their tissues and no parasites were recovered from portions of their spinal cords inoculated on to **cell** cultures. Results of this study demonstrate that **merozoites** of the SN2 isolate of S. neurona will induce seroconversion but not clinical disease when inoculated directly into the CSF of nonimmune horses.
Tags: Female; Male; Research Support, Non-U.S. Gov't
Descriptors: *Encephalomyelitis--veterinary--VE; *Horse Diseases --parasitology--PS; * **Sarcocystis** --pathogenicity--PY; * **Sarcocystosis** --veterinary--VE; Animals; Antibodies, Protozoan--blood--BL; Antibodies, Protozoan--cerebrospinal fluid--CF; Blotting, Western--veterinary--VE; Encephalomyelitis--blood--BL; Encephalomyelitis--cerebrospinal fluid--CF; Encephalomyelitis--parasitology--PS; Enzyme-Linked Immunosorbent Assay --veterinary--VE; Equidae; Horses; Injections, Spinal--veterinary--VE; **Sarcocystosis** --blood--BL; **Sarcocystosis** --cerebrospinal fluid--CF; **Sarcocystosis** --parasitology--PS
CAS Registry No.: 0 (Antibodies, Protozoan)
Record Date Created: 20001103
Record Date Completed: 20001103

10/9/59 (Item 1 from file: 129)
DIALOG(R)File 129:PHIND(Archival)

b129

00731140
Fort Dodge's EPM **(equine protozoal myeloencephalitis) vaccine licence renewed:**
Animal-Pharm 480 p20, November 02, 2001 (20011102)
STORY TYPE: B WORD COUNT: 174

The US Department of Agriculture (USDA)'s Center for Veterinary Biologics has renewed the conditional licence for Fort Dodge Animal Health's **Sarcocystis neurona** , killed protozoa **vaccine** for one year. The **vaccine** is intended to help prevent **equine** protozoal myeloencephalitis (**EPM**) by aiding in the prevention of new infections by S **neurona** , the organism that causes **EPM** , the company says. The renewal was given as a result of the USDA's review of information supplied by Fort Dodge describing its progress toward fulfilling the requirements for a full licence. The data submitted included: a report describing challenge model development experiments conducted and in progress; a preliminary report describing studies of the **vaccine** 's ability to induce cell-mediated immune responses; a progress update concerning the planned multi-centre field performance study of the **vaccine** ; and a report updating the long-term safety of the **vaccine** in which field-trial horses were monitored following an annual booster administration. No adverse events were noted in the 392 horses.
ANIMAL PHARM - World Animal Health & Nutrition News FILED 29 October 2001 COPYRIGHT 2001 PJB Publications Ltd

10/9/67 (Item 3 from file: 50)
DIALOG(R)File 50:CAB Abstracts

0008241943 CAB Accession Number: 20023102736
Seroprevalence of Neospora , Toxoplasma gondii and Sarcocystis **neurona antibodies in horses from Jeju island, South Korea.**
Gupta, G. D.; Lakritz, J.; Kim JaeHoon; Kim DaeYong; Kim JinKap; Marsh, A. E.
Author email address: marshae@missouri.edu
Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Connaway Hall, 1600 East Rollins Dr., Columbia, MO 65211, USA.
Veterinary Parasitology vol. 106 (3): p.193-201
Publication Year: 2002
ISSN: 0304-4017
Digital Object Identifier: 10.1016/S0304-4017(02)00064-X
Publisher: Elsevier Science B.V. Amsterdam, Netherlands
Language: English Record Type: Abstract
Document Type: Journal article
Parasite-specific antibody responses to Neospora spp. and Toxoplasma gondii **antigens** were detected using the indirect fluorescent antibody test (IFAT) and immunoblot analysis in 191 Thoroughbred horses (108 males and 83 females, 1.5-2 years old) from Jeju island, South Korea [date not given]. For comparison, a naturally infected Neospora hughesi horse and an experimentally inoculated T. gondii equid (pony) were used. In addition, all samples were tested for antibodies to **Sarcocystis** neurona by immunoblot analysis. A total of 191 serum samples from clinically normal horses were evaluated. Only 2% (4 out of 191) and 2.6% (5 out of 191) of the samples had showed reactivity at 1:100 using the IFAT for Neospora

spp. and T. gondii , respectively. For T. gondii , two samples matched the **antigen** banding pattern of the positive control by immunoblot analysis. No sample was positive for N. hughesi by immunoblot analysis in this study. Overall, there was a 1% seroprevalence for T. gondii antibodies in the horses tested based on immunoblot analysis. The seroprevalence for S. **neurona** and N. hughesi antibodies was 0%. We concluded that these horses are either not routinely exposed to these parasites or the antibody titres are not sufficiently elevated to be detectable. It is most likely the former explanation since Jeju island **equine** farms are isolated from the main land, and the horses were all less than 3 years of age. This naive population of horses could be useful when evaluating S. **neurona** serodiagnostic tests or evaluating potential S. **neurona** **vaccines** since exposure risks to S. **neurona** and closely related parasites are negligible.
42 ref.

DESCRIPTORS: disease prevalence; neosporosis; **sarcocystosis** ; seroprevalence; Thoroughbred; toxoplasmosis
IDENTIFIERS: Neospora hughesi
ORGANISM DESCRIPTORS: horses; Neospora; **Sarcocystis** neurona; Toxoplasma gondii
GEOGRAPHIC NAMES: Korea Republic
BROADER TERMS: Equus; Equidae; Perissodactyla; mammals; vertebrates; Chordata; animals; ungulates; East Asia; Asia; Developing Countries; Threshold Countries; OECD Countries; **Sarcocystidae** ; Eucoccidiorida; Apicomplexa; Protozoa; invertebrates; Neospora; **Sarcocystis** ; Toxoplasma
CABICODES: Protozoan, Helminth, Mollusc and Arthropod Parasites of Animals, (New March 2000) (LL822)
? logoff hold
>>>KWIC option is not available in file(s): 399

10/3,KWIC/6 (Item 1 from file: 654)
DIALOG(R)File 654:US Pat.Full.

5993734
Derwent Accession: 2005-161937
UTILITY
Detection of sarcocystis **neurona**
Inventor: Dame, John B., Gainesville, FL, US
Ellison, Siobhan P., Fairfield, FL, US
Yowell, Charles A., Gainesville, FL, US
Assignee: Unassigned
Correspondence Address: SALIWANCHIK LLOYD & SALIWANCHIK;A PROFESSIONAL ASSOCIATION, PO BOX 142950, GAINESVILLE, FL, 32614-2950, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20050037443	A1	20050217	US 2004916046	20040810
Division	US 6808714			US 2001962993	20010924
Provisional				US 60-234676	20000922

Fulltext Word Count: 19644
Detection of sarcocystis **neurona**

Abstract:

[00000] A gene encoding a 29 kilodalton **protein** found on the surface of **merozoite** stage S. **neurona** has been cloned and sequenced. The **protein** encoded by this gene, termed SnSAG-1, is an immunodominant **antigen** recognized on **protein** blots. Methods for using nucleic acids and polypeptides relating to SnSAG-1 in diagnostic tests and **vaccine** development are disclosed.

Summary of the Invention:

...microbiology and veterinary medicine. More particularly, the invention concerns compositions and methods relating to detecting **Sarcocystis** neurona...

...0004] **Equine** Protozoal Myeloencephalitis (**EPM**) is a common cause of neurologic disease in New World horses. It is caused by a parasite termed **Sarcocystis** neurona (S. neurona), an obligatory intracellular apicomplexan parasite whose multi-phase life cycle is completed...

...a horse has been infected, S. neuronacan travel to the brain and spinal cord, where **merozoite** stages of this parasite replicate and cause pathology0005] Horses with **EPM** typically present with lameness, but may alternatively or additionally present with symptoms characteristic of primary...

...inhabit any area of the central nervous system (CNS) of the horse, symptoms associated with **EPM** can vary widely. The degree of infection can range from subtle to severe and can involve the brain and/or the spinal cord. **EPM** is usually progressive...

...0006] Presently, a definitive diagnosis of **EPM** is made by post-mortem examination, where S. neurona organisms are identified in histological lesions...

...or when cultured from the lesion establishes the diagnosis. Heretofore, pre-mortem methods for diagnosing **EPM** were based on assays using whole **merozoites** , and not a purified **protein** , to probe for the presence of anti-S. neurona antibodies (as an indication of infection) in the horses. The use of such whole **merozoites** results in significant cross-reaction with non-S. neurona specific antibodies (e.g., those against other **Sarcocystis** species). This cross-reactivity obscures interpretation of results using whole **merozoite** -based assays...0007] The invention relates to the discovery and characterization of a 29 kilodalton (kDa) **protein** found on the surface of **merozoite** stage S. neurona. This **antigen** , termed SnSAG-1 or SnSMA1, is an immunodominant **antigen** recognized on **protein** blots. Using purified or recombinant SnSAG-1 (i.e., rSnSAG-1) **antigen** , accurate assays for diagnosing **EPM** in horse pre-mortem have been developed. These assays involve identifying a marker indicative of the presence of the 29 kDa **antigen** or an antibody to this **antigen** in a sample to be tested. Thus, because a single purified **antigen** or marker is utilized in such assays, the cross-reactivity problems associated with whole-merozite...

...0008] A cDNA copy of the mRNA which encodes the SnSAG-1 **antigen** has been cloned from a gene library prepared from an isolate of S. **neurona** . The original clone was identified in a collection of random sequence tags prepared to characterize...determined. This sequence or the clone itself can be used to prepare the SnSAG-1 **antigen** in a recombinant or other

synthetic form for use in diagnostic tests and **vaccine** development...

...0009] Accordingly, the invention features a composition for detecting the presence of S. **neurona** in a biological sample. In one variation, the composition includes a SnSAG-1 marker that is a purified nucleic acid including a nucleotide sequence that encodes a **protein** that shares at least 50% or at least 90% sequence identity with SEQ ID NO: 1. In this variation, the nucleotide sequence can also encode the **protein** of SEQ ID NO: 1. For example, the nucleotide sequence can be SEQ ID NO...0011] In a third variation of the composition, the SnSAG-1 marker is an isolated **protein** including a polypeptide that shares at least 50%, 70%, 90%, or 95% sequence identity with...

...include the entire amino acid sequence of SEQ ID NO: 1. In this composition, the **protein** can be a fusion **protein** or a recombinant **protein** .

[0013] In another aspect, the invention features a method for detecting **Sarcocystis** neurona in a biological sample. This method includes the steps of: (a) providing the biological...

...SnSAG-1 marker that is a nucleic acid including a nucleotide sequence that encodes a **protein** that shares at least 50% sequence identity with SEQ ID NO: 1; a polynucleotide that...

...ID NO:3, wherein the polynucleotide can be at least 30 nucleotides in length; a **protein** including a polypeptide that shares at least 50% sequence identity with a fragment of the...
...of the SnSAG-1 marker in the biological sample indicates that the biological sample contains **Sarcocystis** neurona...

...the SnSAG-1 marker is a nucleic acid including a nucleotide sequence that encodes a **protein** that shares at least 50% sequence identity with SEQ ID NO: 1, the nucleotide sequence

Description of the Invention:
...0037] A gene encoding a S. neurona surface **antigen** has been cloned and sequenced. The **antigen** encoded by the gene has been characterized. Rabbit anti-S. neurona polyclonal antibody was used to immunoprecipitate and concentrate **proteins** of an isolate of S. neurona for detection of antibodies in body fluids of clinically...

...horses. The serum and cerebral spinal fluid (CSF) of diseased animals was used to identify **antigens** important in natural infections. Techniques were developed to separate parasites from host **cells** facilitating production a cDNA expression library. The library was screened with both polyclonal rabbit anti-S. neurona and mass culture supernatant from hybridoma **cells** produced from mice immunized with the S. neurona isolate. A cone was also identified in...
...a probe to select the full length copy of the gene encoding a major surface **antigen** , SnSAG-1, of S. neurona. The sequence data from the full length gene was used...BamH1 site of the expression vector pET14b which allows expression of a His-tagged recombinant **protein** (i.e., His-tagged rSnSAG-1). This recombinant **protein** migrated slightly larger on SDS-PAGE than the native **antigen** .

[...

...0039] A monoclonal antibody that specifically binds an epitope of the 29 kDa **protein** (corresponding to SnSAG-1) from cultured S. neurona **merozoites** was used to verify the presence of the epitope on the recombinant **protein** . The recombinant **protein** was purified and used to produce a monospecific polyclonal antibody in mice and goats. The anti-SnSAG-1 antisera was used to characterize the 29 kDa **antigen** of cultured S. neurona **merozoites** as a surface **protein** .

[...be performed, for example, on commercial automated oligonucleotide synthesizers. Immunological methods (e.g., preparation of **antigen** -specific antibodies, immunoprecipitation, and immunoblotting) are described, e.g., in Current Protocols in Immunology, ed...

...SEQ ID NO:2. The region of this nucleic acid encoding the native SnSAG-1 **protein** (see SEQ ID NO:3) is found in positions 73-903 (SEQ ID NO:3... or non-coding (anti-sense) strand. The coding sequence which encodes the native SnSAG-1 **protein** may be identical to the nucleotide sequence shown herein as SEQ ID NO:3. It...gene such as those that encode fragments, analogs and derivatives of a native SnSAG-1 **protein** . Such variants may be, e.g., a naturally occurring allelic variant of the native SnSAG...

...0046] Variant SnSAG-1 **proteins** displaying substantial changes in structure can be generated by making nucleotide substitutions that cause

less...

...an amino acid side chain. Nucleotide substitutions generally expected to produce the greatest changes in **protein** properties are those that cause non-conservative changes in codons. Examples of codon changes that are likely to cause major changes in **protein** structure are those that cause substitution of (a) a ...the native SnSAG-1 gene, and encode polypeptides having structural similarity to native SnSAG-1 **protein** . Homologs of the native SnSAG-1 gene within the invention are nucleic acids isolated from...

...the native SnSAG-1 gene, and encode polypeptides having structural similarity to native SnSAG-1 **protein** . Public and/or ...the native SnSAG-1 gene, and encode polypeptides having structural similarity to native SnSAG-1 **protein** (e.g., those that cross react with antibodies that specifically bind the native SnMS1 **protein**). Examples of non-naturally occurring SnSAG-1 gene variants are those that encode a fragment of a SnSAG-1 **protein** , those that hybridize to the native SnSAG-1 gene or a complement of to the...

...complement of the native SnSAG-1 gene, and those that encode an SnSAG-1 fusion **protein**

Exemplary or Independent Claim(s):

1. A method for detecting **Sarcocystis** neurona in a biological sample, the method comprising the steps of:
(a) providing a biological...

...1, or composition thereof, with a biological sample and detecting the formation of an antibody- **antigen** complex...

...12. A kit for detecting **Sarcocystis** neurona comprising a polypeptide comprising SEQ ID NO: 1 or a polypeptide fragment of SEQ...

10/3,KWIC/9 (Item 4 from file: 654)
DIALOG(R)File 654:US Pat.Full.

0005764804 **IMAGE Available
Derwent Accession: 2004-614854
Nucleic acids encoding Sarcocystis **neurona** antigen **and uses thereof**
Inventor: Howe, Daniel, INV
Correspondence Address: KING & SCHICKLI, PLLC, 247 NORTH BROADWAY, LEXINGTON, KY, 40507, US

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 20040162418	A1	20040819	US 2003369430	20030219

Fulltext Word Count: 18910

Nucleic acids encoding Sarcocystis **neurona** antigen **and uses thereof**

Abstract:
The present invention provides novel isolated nucleic acids encoding **antigenic proteins** derived from **Sarcocystis** neurona, or unique fragments thereof. In particular, the invention provides novel isolated nucleic acids encoding membrane-associated polypeptides SnSAG2, SnSAG3, and SnSAG 4. Also provided are purified **antigenic** polypeptide fragments encoded by the novel nucleic acid sequences set forth herein that encode for **Sarcocystis** neurona. In particular, the invention provides purified **antigenic** polypeptide fragments encoded by the novel nucleic acid sequences set forth herein that encode for...

...Further, the invention provides a purified **antigenic** polypeptide fragment encoded by the nucleic acid sequences set forth herein or a selective portion...

...carrier. Also provided isolated nucleic acids capable of selectively hybridizing with the nucleic acid from **Sarcocystis** neurona. The invention also provides vectors comprising the nucleic acids of the invention encoding **Sarcocystis** neurona or a unique fragment thereof and provides the vector in a host capable of...

...Finally, the invention provides a purified polyclonal and/or a monoclonal antibody specifically reactive with **Sarcocystis** neurona and a method of detection of **Sarcocystis** neurona utilizing the antibodies of the invention...

Summary of the Invention:
...0002] The present Invention relates to nucleic acids of **Sarcocystis neurona** . In particular, the present invention relates to nucleic acids of **Sarcocystis neurona** and to nucleic acid reagents and antibodies for use in methods of detection and prevention of **Sarcocystis neurona** infection. More particularly, the present invention relates to novel nucleic acid sequences of **Sarcocystis neurona** and to utilization thereof including primers, probes, antigen/antibody diagnostic kits, vectors for production of peptides encoding the novel nucleic acids, and to antigenic proteins and **vaccines** against **Sarcocystis neurona** .

...

...0003] **Sarcocystis** neurona is an apicomplexan parasite that is the primary cause of **equine** protozoal myeloencephalitis (**EPM**). Due to several factors, definitive pre-mortem diagnosis of **EPM** remains exceedingly difficult. In particular, the seroprevalence of S. neurona in horses is significant, yet the true incidence of **EPM** is quite low, thus indicating that infection does not equate with disease. Additionally, the immunoblot remains the only commercial assay available for testing samples from suspect **EPM** horses; while development of this test was a significant advance, it is a decade-old...

...0004] **EPM** is a common and debilitating infectious disease that affects the central nervous system of horses...

...Rooney et al., 1970), but it was not until 1991 that the etiological agent of **EPM** was isolated and designated S. neurona (Dubey et al., 1991). S. neurona is related to...

...during growth in the intestinal epithelium of their definitive host. Similar to other species of **Sarcocystis** , S. neurona has an obligatory heteroxenous life cycle, with the opossum (Didelphis virginiana) serving as...by ingesting sporocysts in feces from the opossum, but unlike the normal intermediate hosts, mature **sarcocysts** have not been found in **equine** tissues (MacKay et al., 2000); consequently, the horse is currently considered an aberrant dead-end...

...The geographic range of S. neurona appears to be limited to the Western Hemisphere, thus **EPM** primarily affects horses in the Americas...

...1997), suggesting that these animals are commonly exposed to the parasite. However, the incidence of **EPM** is estimated to be below 1% (MacKay et al., 2000), indicating that there is a...

...S. neurona sporocysts gave inconsistent results, and these studies were unable to authentically reproduce acute **EPM** (Cutler et al., 2001; Fenger et al., 1997). Consequently, it is apparent that other factors...

...infection are responsible for the progression to disease. It is well established that a robust **cell** -mediated immune response is important for controlling infections by coccidian parasites (Alexander et al., 1997 al., 2001a; Marsh et al., 1997), and it is possible that susceptibility to **EPM** may be increased in horses with inappropriate and/or suppressed immune responses during S. neurona...

...stress to induce a transient immunosuppression has been shown to provide some improvement to the **equine** challenge model for **EPM** (Saville et al., 2001...

...0006] Definitive antemortem diagnosis of **EPM** remains exceedingly difficult, for a variety of reasons. Horses afflicted with **EPM** exhibit signs that are similar to a number of different neurological disorders (MacKay et al...

...not equate to disease, since only a small proportion of seropositive horses will suffer from **EPM** ; as a consequence, the detection of anti-S. neurona antibodies in serum provides little diagnostic...

...antibody production, thus suggesting CNS infection, has improved the predictive value of antibody detection for **EPM** diagnosis. However, interpretation of CSF antibody presence can be confounded by contamination of the CSF...

...assays are hampered by several intrinsic problems, and they provide only mediocre predictive value for **EPM** diagnosis. Western blot analysis (a.k.a., immunoblot) of crude S. neurona lysate remains the immunodiagnostic test that is used to detect antibodies in suspect **EPM** horses (Granstrom et al., 1993). The continued use of the immunoblot has been necessitated by perceived **antigenic** cross-reactivity between different species of **Sarcocystis** , and the assay relies on the recognition of several **antigens** , primarily in the low molecular weight range, by serum/CSF antibodies (Dubey et al., 2001b...

...to improve the immunoblot test have included the use of antibodies against the related parasite **Sarcocystis** cruzi to block cross-reactive epitopes, theoretically increasing the specificity of the immunoblot analysis for...

...value. While the immunoblot has been utilized for a number of years to help diagnose **EPM** , it is a first-generation test that needs to be replaced with improved assays based...0007] Research efforts directed toward understanding immunity against S. neurona infection and improving **EPM** diagnosis have been somewhat hampered by the lack of molecular information for S. neurona. The identification of S. neurona-specific **antigens** and characterization of the genes encoding these **antigens** as provided by the present invention hereby allow for the production of recombinant parasite **antigens** via expression in E. coli and the subsequent generation of monoclonal and monospecific polyclonal antibodies against the individual S. neurona **antigens** . The recombinant **proteins** and specific antibodies provided by the invention serve as valuable reagents for conducting immunological studies on S. neurona infections and the progression to **EPM** . Additionally, these reagents allow for the development of new and more reliable diagnostic tests; for example, a recombinant S. neurona **antigen** furnishes the key component for a simple and efficient enzyme-linked immunosorbent assay (ELISA) that can be used to monitor specific antibodies in **equine** serum or CSF. As provided by the teachings herein, the development of an ELISA that is based on a single recombinant S. neurona **antigen** rather than whole-parasite lysate provides a second-generation assay that significantly improves current methodologies for identifying S. neurona-infected animals. Notably, the use of a single **antigen** ELISA will allow for a more in-depth and complete dissection of antibody responses to infected and suffering from **EPM** .

[...

...0008] A fluctuating equilibrium is maintained between the **cell** -mediated and the humoral (antibody) responses of the vertebrate immune system, and this balance will...

...in their profile of secreted cytokines, and these immune factors target and regulate different effector **cells** and mechanisms. Immunoglobulin isotype switching is an important immune mechanism that allows the host to...

...Finkelman et al., 1990; Snapper et al., 1997). It is generally believed

that a Th1 **cell** -mediated response is necessary for control of coccidian parasites (Alexander et al., 1997; Krahenbuhl and...nature of the immune response (i.e., Th1 versus Th2) in S. neurona-infected and **EPM** horses. The selection of an **antigen** for development of a diagnostic test can be somewhat subjective since any particular pathogen is composed of numerous **antigenic proteins** . Logically, the target molecule in a diagnostic assay must unfailingly elicit a detectable antibody response in the infected animal. A number of previous studies have demonstrated that surface **antigens** of the Coccidia are exceedingly immunogenic. In particular, the primary surface **antigens** of Toxoplasma gondii (Handman and Remington, 1980; Sharma et al., 1983) and Neaspora caninum (Howe et al., 1998) have been shown to be immunodominant. These surface **antigens** , designated SAGs and SAG-related sequences (SRSs), have been implicated in host **cell** attachment and invasion by the parasite (Dzierszinski et al., 2000; Grimwood and Smith, 1992; Hemphill...

...1994; Mineo et al., 1993), most likely through interactions with sulfated proteoglycans on the host **cell** surface (He et al., 2002; Jacquet et al., 2001). In addition to their probable role as adhesins, there is increasing evidence that some of these surface **antigens** are involved in modulation of the host immune response (Lekutis et al., 2001). Significantly, the TgSAG1 surface **antigen** of T. gondii has been shown to protect mice against acute toxoplasmosis (Bulow and Boothroyd, 1991), and the NcSAG1 (p29) major surface **antigen** of N. caninum has been used to develop an ELISA for detection of Neospora infection...
...et al., 2002). Collectively, these previous studies demonstrate that coccidian SAGs are at least candidate **proteins** for the development of both diagnostic assays and protective **vaccines** . Prior to the present invention, however, it had not been shown that the surface **antigens** of S. **neurona** (i.e., ...target molecules for examining immune responses in infected horses and for developing improved assays for **EPM** diagnosis. The present invention utilizes recombinant S. **neurona** SAGs that are provided by the invention to provide simple and reliable ELISAs, and these assays can be used to scrutinize specific humoral immune responses in **EPM** horses and for detecting the presence of S. **neurona** in a test sample. Importantly, the developed ELISAs are valuable as tools to aid in the diagnosis of **EPM** infection in horses...

...0009] Nucleic acids of certain **Sarcocystis** and Toxoplasma species are known in the art. For example, Eschenbacher K-H et al. "Cloning and expression in Escherichia coli of cDNAs encoding a 31-kilodalton surface **antigen** of **Sarcocystis** muris". Molec. Biochem. Parasitol. 1992, 53:159-168 (1992). Eschenbacher discloses the cloning and expression of a surface coat **protein** of **Sarcocystis** muris **merozoites** consisting of 280 amino acids with a predicted size of 31 kDa...

...0010] Velge-Roussel F. et al. "Intranasal Immunization with Toxoplasma gondii SAG1 induces protective **cells** into both NALT and GALT compartments. Infection and Immunity, 2000, 68: 969-972, discloses that intra-nasal immunization with a SAG1 **protein** derived from ...this case the mouse, and can be partially controlled by i.n. immunization with the **protein** SAG1 plus CT...

...discloses the construction of a DNA vaccine using the recombinant form of the surface coat **protein** SAG1 in Toxoplasma gondii, consisting of 824-nucleotides encoding the 275 amino acid **protein** . Animals immunized with this plasmid produce anti-SAG1 antibodies which recognize the native

SAG1. See...Toxoplasma gondii SAG1 used in vaccination had a significant protective effect against maternofetal transmission of **tachyzoites**. Absence of parasites in fetuses was demonstrated in 66-86% of fetuses from adult guinea...V, Bollen A, Beaumans R, Jacquet A. "Protective immunity against congenital toxoplasmosis with recombinant SAG1 **protein** in a guinea pig model". Infect Immun. 2000 September;68(9):4948-53...

...0015] Angus et al. discloses that immunization with a DNA plasmid encoding the SAG1 (p30) **protein** of Toxoplasma gondii is immunogenic and protective in mice. Sera of immunized mice showed recognition of T. gondii **tachyzoites** by immunofluorescence and exhibited high titers of antibody to SAG1 by ELISA. This data suggest...
...Dubey J P, Kovacs J A." Immunization with a DNA plasmid encoding the SAG1 (P30) **protein** of Toxoplasma gondii is immunogenic and protective in rodents". J Infect Dis. 2000 January;181...

...0016] Fort Dodge Animal Health, " **Vaccine** Development" discloses that an S. **neurona** merozoite culture that is chemically inactivated and incorporates an adjuvant is used as an **EPM** **vaccine** . This **vaccine** has been conditionally licensed for use but without any indication of its effectiveness in preventing Sarcocyst **neurona** induced **EPM** Fort Dodge Animal Health, " **Vaccine** Development" Discloses that an S. **neurona** merozoite culture that is chemically inactivated and incorporates an adjuvant is used as the **EPM** **vaccine** . Fort Dodge Animal Health, 20001 ...

...4):289-310; O,Donoghue P J et al. "Attempted immunization of swine against acute **sarcocystosis** using cystozooite-derived **vaccines** ". Vet. Immunol Immunopathol. 1985 January;8(1-2):83-92; Bulow R and Boothroyd J. C. "Protection of mice from fatal Toxoplasma gondii infection by immunization with p30 **antigen** in liposomes". J. Immunol. 1991, 147 3496-3500; Dame J B, MacKay R J, Yowell...

...Marsh A, Greiner E C "S. falcatula from passerine and psittacine birds: synonymy with S. **neurona** , agent of **EPM** ". J. Parasitol. 1995, December; 81(6):930-5; Mishima M, Xuan X, Shioda A, Omata...

...H, Mikami T. "Modified protection against Toxoplasma gondii lethal infection and brain cyst formation by **vaccination** with SAG2 and SRS1". J Vet Med Sci. 2001 April;63(4):433-8; Aosai...

...Hata H, Kobayashi M, Kiuchi M, Stauss H J, Yano A. "Protective immunity induced by **vaccination** with SAG1 gene-transfected **cells** against Toxoplasma gondii infection in mice". Microbiol Immunol. 1999;43(1):87-91; Artois M...the foregoing art, there remains a need in the art for a safe and effective **vaccine** against **Sarcocystis** **neurona** . Likewise, as set forth above there is also a need in the art for diagnostic kits including **antigen** and antibody kits for fast and reliable diagnosis of **Sarcocystis** **neurona** infection of encoding **antigenic** **proteins** derived from **Sarcocystis** neurona, or unique **antigenic** fragments thereof. It is also an object of the present invention to provide purified **antigenic** polypeptide fragments encoded by the novel nucleic acid sequences set forth herein that encode for **Sarcocystis** neurona. In particular, it is an object of the present invention to provide a purified **antigenic** polypeptide fragment encoded by the nucleic acid sequences set forth herein or a selective portion...
...invention to provide isolated nucleic acids capable of selectively

hybridizing with the nucleic acid from **Sarcocystis** neurona including, but not limited to, primers and probes for utilization in polymerase chain reaction...

...Another object of the invention is to provide a vector comprising the nucleic acid encoding **Sarcocystis** neurona or a unique fragment thereof and to provide the vector in a host capable...object of the invention is to provide a purified antibody that is selectively reactive with **Sarcocystis** neurona or an immunodominant polypeptide provided by the invention or a genetic variant thereof. A...

...object of the present invention is to provide a purified monoclonal antibody specifically reactive with **Sarcocystis** neurona and a method of detection of **Sarcocystis** neurona utilizing the antibodies of the present invention...

...satisfies the need in the art by providing a novel isolated nucleic acid encoding an **antigenic protein** derived from **Sarcocystis** neurona, or a unique fragment thereof. In one embodiment, the invention provides novel isolated nucleic...

...0024] The present invention also provides purified **antigenic** polypeptide fragments encoded by the novel nucleic acid sequences set forth herein that encode for **Sarcocystis** neurona. In one embodiment, the invention provides purified **antigenic proteins** or purified **antigenic** polypeptide fragments encoded by the novel nucleic acid sequences set forth herein that encode for SnSAG2, SnSAG3, and SnSAG 4. In another embodiment, the present invention provides a purified **antigenic** polypeptide fragment encoded by the nucleic acid sequences set forth herein or a selective portion...

...invention also provides isolated nucleic acids capable of selectively hybridizing with the nucleic acid from **Sarcocystis** neurona including, but not limited to, primers and probes for utilization in polymerase chain reaction...

...Further, the present invention provides vectors comprising the isolated nucleic acids set forth herein encoding **Sarcocystis** neurona or a unique fragment thereof and provides the vector in a host capable of...present invention also provides a purified polyclonal and or a monoclonal antibody specifically reactive with **Sarcocystis** neurona and a method of detection of **Sarcocystis** neurona utilizing the antibodies of the present invention

Description of the Drawings:

...is a sequence comparison of SnSAG1, SnSAG3, and SnSAG4 with TgSAG2E. The S. neurona surface **antigens** SnSAG1, SnSAG3 and SnSAG4 are most similar to the TgSAG2 family of T. gondi surface **antigens** . Sequence alignments of the predicted mature **proteins** revealed very moderate sequence identity (<25%). However, the SnSAGs contain 10/12 conserved cysteine residues...

...2 is a sequence comparison of SnSAG2 with TgSAG1 and TgSRS2. The S. neurona surface **antigen** SnSAG2 is most similar to the TgSAG1 family of T. gondii surface **antigens** . Similar to the other SnSAGs, SnSAG2 shares modest sequence identity to its TgSAG orthologues, but...0030]FIG. 3 shows a Western blot analysis of the Sn SAGs in S. neurona **merozoites** . The SnSAG genes were expressed in E. coli, and monospecific polyclonal antisera were generated against the recombinant **proteins** . Western blot

analysis of reduced **antigen** revealed that each SnSAG migrated significantly higher than its predicted molecular weight, consistent with what...

...0031]FIG. 4 shows the SnSAGs are membrane-associated in **Sarcocystis** neurona merozites. Triton X-114 partitioning assays indicated that the SnSAGs are associated with membranes, consistent with their surface localization via glycolipid anchoring. Western blot analysis of the partitioned **proteins** with the SnSAG-specific polyclonal antisera revealed that all four SnSAGs were separated exclusively into the detergent phase (D). The control **protein** , SnMIC10, was partitioned into the aqueous phase (A), as expected

Description of the Invention:

...long felt need in the art by providing novel isolated nucleic acid sequences which encode **antigenic** **proteins** derived from **Sarcocystis** neurona, or which encode unique **antigenic** **protein** fragments thereof. As used herein, a "nucleic acid" means a chain of at least two...

...nucleic acid is one that is substantially separated from other nucleic acid sequences in a **cell** or organism in which the nucleic acid naturally occurs. Likewise, by "isolated" nucleic acid is...

...and are meant to include genomic and subgenomic nucleic acids found in the naturally-occurring **Sarcocystis** neurona organism. The nucleic acids contemplated by the present invention include a nucleic acid having sequences from which a **Sarcocystis** neurona cDNA can be transcribed; or allelic variants and/or homologs of thereof. By "capable...

...hybridize with other nucleic acids to prevent an adequate positive hybridization with nucleic acids from **Sarcocystis** neurona and is meant to include stringent hybridization conditions including low, moderate and high stringency...the novel sequences set forth herein or that can selectively hybridize with nucleic acids from **Sarcocystis** neurona. Modifications to the nucleic acids of the invention are also contemplated as long as...

...In particular, one embodiment of the present invention provides isolated nucleic acid derived from three **Sarcocystis** neurona cluster sequences, namely Sn Cluster 144, Sn Cluster 21 and Sn Cluster 4, which...29 respectively and the sequences complimentary thereto. Also provided by the invention are the corresponding **protein** or polypeptide amino acid sequences for these three **Sarcocystis** neurona cluster sequences. The polypeptide sequence comprising Sn Cluster 144 is set forth in the...

...the Sequence Listing as SEQ ID NO: 30. As used herein, the terms "polypeptide" and " **protein** " are used interchangeably and are meant to include any peptide-linked chain of amino acids...

...a polypeptide that has been substantially separated or isolated away from other polypeptides in a **cell** , organism, or mixture in which the polypeptide occurs...

...0036] **Sarcocystis** neurona is an apicomplexan parasite that can cause a severe neurologic disease in horses called **equine** protozoal myeloencephalitis (**EPM**). Similar to other members of the Apicomplexa, S. neurona is an obligate intracellular pathogen that...

...novel and undoubtedly important since they are responsible for the

initial interactions with the host **cell** surface and host immune response. In Toxoplasma gondii for example, an extensive family of 25+ surface **antigens** has been identified, which are developmentally regulated and exhibit various levels of sequence similarity to either of the major T. gondii surface **antigens** TgSAG1 or TgSAG2. These surface molecules appear to be involved in receptor/ligand interactions with the host **cell** surface, and there is increasing evidence that some of the T. gondii SAGs are involved...

...present invention provides four isolated nucleic acids of S. neurona (genes) that encode parasitic surface **antigens** . A sequencing project was conducted that generated approximately 8500 expressed sequence tags (ESTs) from this...

...of this sequence database has revealed a family of at least four S. neurona surface **antigens** that are orthologues of the SAG/SRS family of surface **proteins** in T. gondii. Based on their homology to the T. gondii SAGs, the novel S. neurona surface **antigens** have been designated SnSAG1, SnSAG2, SnSAG3, and SnSAG4 respectively. Each **protein** is predicted to contain an amino-terminal signal peptide and a carboxyl-terminal glycolipid anchor...

...surface localization, and Triton X-114 partitioning and surface biotinylation assays confirmed that all four **proteins** are membrane-associated and displayed on the S. neurona **merozoite** surface (See, FIGS. 4 and 5). Additionally, these novel S. neurona **proteins** possess multiple conserved cysteine residues that have been described previously for T. gondii SAGs and which are likely important for the tertiary structure of the **proteins** (See, FIGS. 1 and 2). Due to their surface localization and relative homology to T. gondii surface **antigens** , these S. neurona **proteins** have been designated SnSAG1, SnSAG2, SnSAG3, and SnSAG4...

...ID NO: 21 comprises an 828-nucleotide open reading frame of the SnSAG1 gene of **Sarcocystis** neurona which encodes a 276 amino acid polypeptide set forth in the Sequence Listing as...

...addition site at the carboxy-terminal end (indicating surface localization). Database searches with the predicted **protein** sequence of SnSAG1 (rSnSAG1) revealed significant similarity (alignment score=80, E value=2X10-14) to a 31 kDa surface **antigen** from **Sarcocystis** muris...

...0039] A recombinant form of the **Sarcocystis** neurona SnSAG1 (rSnSAG1) has been expressed in E. coli. Western blot analysis of rSnSAG1 demonstrated that the recombinant **antigen** is recognized by antiserum from a rabbit that was immunized with S. neurona **merozoites** and by antibodies in cerebrospinal fluid (CSF) from an **EPM** (**Sarcocystis** neurona infected) horse (See, e.g., FIG. 3...

...ID NO: 23 comprises an 975 nucleotide open reading frame of the SnSAG2 gene of **Sarcocystis** neurona which encodes a 168 amino acid polypeptide set forth in the Sequence Listing as...

...ID NO: 25 comprises an 1585 nucleotide open reading frame of the SnSAG2 gene of **Sarcocystis** neurona which encodes a 281 amino acid polypeptide set forth in the Sequence Listing as...ID NO: 27 comprises an 1111 nucleotide open reading frame of the SnSAG2 gene of **Sarcocystis** neurona which encodes a 287 amino acid polypeptide set forth in the Sequence Listing as...

...0043] As set forth more fully below, these genes have been expressed as recombinant **proteins** in E. coli. The recombinant SnSAG **proteins** can be implemented into antibody-capture ELISAs and used to detect the presence of S. **neurona** antibodies in a sample. Likewise, the recombinant **proteins** provided by the invention can be used as reagents for use in **vaccines** against S. **neurona** .

[...

...present invention includes the discovery of additional novel expressed sequence tags (EST) that encode novel **antigenic** peptides for utilization in the **vaccines** and diagnostic kits as disclosed by this invention...0045] In particular, in a presently preferred embodiment of the invention, cluster analysis of the **Sarcocystis neurona** expressed sequence tags (ESTs) generated from the cSn.1 cDNA library has revealed a gene family that encodes at least eight homologous **proteins** . Of the approximately 8500 S. neurona ESTs that have been generated thus far, roughly 540 sequences can be placed in this gene family, which has been provisionally designated SnGF1 (S. **neurona** Gene Family 1). Based on its relative abundance in the collection of S. **neurona** ESTs, SnGF1 encodes a set of similar **proteins** (at least eight) that are highly expressed and most likely play significant roles in the biology of S. **neurona** (i.e., parasite virulence factors). In addition to their biological importance, the abundance of these **proteins** would suggest that they elicit significant immune responses in infected animals. Collectively, the characteristics of the novel nucleic acids of SnGF1, and the encoded **proteins** therefrom, make this gene family well suited for the development of improved diagnostics and/or **vaccines** for **EPM** as set forth herein...

...isoforms identified thus far have been designated SnGF1a-h. These genes are predicted to encode **proteins** of, e.g., 109 amino acids, 106 amino acids, and 107 amino acids in length, and the **proteins** share approximately 70% to 80% sequence identity. These **proteins** have a predicted N-terminal signal peptide ...acid reagents derived therefrom which can be utilized to diagnose and prevent infection of S. **neurona** . Purified polypeptides encoded by the nucleic acids are also provided. These polypeptides can be utilized in methods of diagnosis or as **vaccine** components for prevention of infection. Vectors are also provided which comprise the nucleic acids of the present invention. The vectors can be utilized in host expression systems to produce **antigenic** peptide reagents for diagnostic and prophylactic applications. The present invention also provides purified antibodies selectively reactive with S. **neurona** . These antibodies can be used in various diagnostic methods or as a therapeutic...

...0056] In one embodiment, the invention provides purified **antigenic** polypeptides encoded by the nucleic acids set forth in the Sequence Listing. The invention also provides these **antigenic** polypeptides in a pharmaceutically acceptable carrier. The amino acid sequence of these polypeptides can be...

...0057] Purified **antigenic** polypeptide fragments encoded by the nucleic acids of the present invention are also contemplated. As used herein, "purified" means the **antigen** is at least sufficiently free of contaminants or **cell** components with which the **antigen** normally occurs to distinguish the **antigen** from the contaminants or components.

Purified **antigenic** polypeptides of S. neurona and **antigenic** fragments thereof of the present invention are also referred to herein as "the **antigen** " or "the S. neurona **antigen** ." It is contemplated that the **antigenic** fragments can be encoded from any portion of the nucleic acid encoding S. neurona as...

...24, 26 and 28 as described herein. Specifically, one example provides an approximately 12 kDa **antigenic** polypeptide encoded by an open reading frame of SEQ ID NO: 24 consisting essentially of...

...0058] An **antigenic** fragment of the **antigen** can be isolated from the whole **antigen** by chemical or mechanical disruption. The purified fragments thus obtained can be tested to determine their **antigenicity** and specificity by the methods taught herein. **Antigenic** fragments of the **antigen** can also be synthesized directly. An immunoreactive fragment is generally an amino acid sequence of at least about five consecutive amino acids derived from the **antigen** amino acid sequence 0059] The polypeptide fragments of the present invention can also be recombinant **proteins** obtained by cloning nucleic acids encoding the polypeptide in an expression system capable of producing the **antigenic** polypeptide or fragments thereof...

...0060] Once the amino acid sequence of the **antigen** is provided, it is also possible to synthesize, using standard peptide synthesis techniques, peptide fragments chosen to be homologous to immunoreactive regions of the **antigen** and to modify these fragments by inclusion, deletion or modification of particular amino acids residues...

...sequences. Thus, synthesis or purification of an extremely large number of peptides derived from the **antigen** is possible...

...acid sequences of the present polypeptides can contain an immunoreactive portion of the S. neurona **antigen** attached to sequences designed to provide for some additional property, such as solubility. The amino acid sequences of an S. neurona **antigen** can include sequences in which one or more amino acids have been substituted with another...administered to an animal and the immunological response (e.g., the production of antibodies or **cell** mediated immunity) of an animal to each concentration is determined. The amounts of **antigen** administered depend on the subject, e.g. a horse or a guinea pig, the condition...

...the subject, the size of the subject, etc. Thereafter an animal so inoculated with the **antigen** can be exposed to the parasite to test the potential vaccine effect of the specific...

...other fluids or lymphocytes from the inoculated animal for cross reactivity with other closely related **Sarcocystis** spp...

...provided. The vectors of the invention can be in a host capable of expressing the **antigenic** polypeptide fragments contemplated ...known to one of ordinary skill in the art useful for the expression of the **antigen** . Other microbial hosts suitable for use include bacilli, such as Bacillus subtilis, and other enterobacteriaceae...

...also make expression vectors, which will typically contain expression control sequences compatible with the host **cell** (e.g., an origin of replication). In addition, any number of a variety of well...

...can be provided by insertion of a Met codon 5' and in-frame with the

antigen . Also, the carboxyterminal extension of the **antigenic** fragments can be removed using standard oligonucleotide mutagenesis procedures...

...can be used. There are several advantages to yeast expression systems. First, evidence exists that **proteins** produced in a yeast secretion systems exhibit correct disulfide pairing. Second, post-translational glycosylation is...

...factor leader region (encoded by the MF.alpha.-1 gene) is routinely used to direct **protein** secretion from ...sequence for a yeast protease encoded by the KEX2 gene: this enzyme cleaves the precursor **protein** on the carboxyl side of a Lys-Arg dipeptide cleavage-signal sequence. The **antigen** coding sequence can be fused in-frame to the pre-pro-alpha-factor leader region...

...strong transcription promoter, such as the alcohol dehydrogenase I promoter or a glycolytic promoter. The **antigen** coding sequence is followed by a translation termination codon which is followed by transcription termination signals. Alternatively, the **antigen** coding sequences can be fused to a second **protein** coding sequence, such as Sj26 or .beta.-galactosidase, used to facilitate purification of the fusion **protein** by affinity chromatography. The insertion of protease cleavage sites to separate the components of the fusion **protein** is applicable to constructs used for expression in yeast...

...0065] Mammalian **cells** permit the expression of **proteins** in an environment that favors important post-translational modifications such as folding and cysteine pairing, addition of complex carbohydrate structures, and secretion of active **protein** . Vectors useful for the expression of **antigen** in mammalian **cells** are characterized by insertion of the **antigen** coding sequence between a strong viral promoter and a polyadenylation signal. The vectors can contain genes conferring either gentamicin or methotrexate resistance for use as selectable markers. The **antigen** and immunoreactive fragment coding sequence can be introduced into a Chinese hamster ovary **cell** line using a methotrexate resistance-encoding vector. Presence of the vector DNA in transformed **cells** can be confirmed by Southern analysis and production of a cDNA or opposite strand RNA corresponding to the **antigen** coding sequence can be confirmed by northern analysis. A number of other suitable host **cell** lines capable of secreting intact **proteins** have been developed in the art, and include the CHO **cell** lines, HeLa **cells** , myeloma **cell** lines, Jurkat **cells** , etc. Expression vectors for these **cells** can include expression control sequences, such as an origin of replication, a promoter, an enhancer...

...The vectors containing the nucleic acid segments of interest can be transferred into the host **cell** by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic **cells** , whereas calcium phosphate treatment or electroporation may be used for other celluar hosts...

...0066] Alternative vectors for the expression of **antigen** in mammalian **cells** , those similar to those developed for the expression of human gammainterferon, tissue plasminogen activator, clotting Factor VIII, hepatitis B virus surface **antigen** , protease Nexinl, and eosinophil

major basic **protein** , can be employed. Further, the vector can include CMV promoter sequences and a polyadenylation signal available for expression of inserted nucleic acid in mammalian **cells** (such as COS7 ...

...e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those **cells** transformed with the desired nucleic acid sequences (see, e.g., U.S. Pat. No. 4...is also provided. The antibodies can be specifically reactive with a unique epitope of the **antigen** or they can also react with epitopes of other organisms. The term "reactive" means capable of binding or otherwise associating non randomly with an **antigen** . "Specifically reactive" as used herein refers to an antibody or other ligand that does not cross react substantially with any **antigen** other than the one specified, in this case, S. neurona. Antibodies can be made as...

...A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1988). Briefly purified **antigen** can be injected into an animal in an amount and in intervals sufficient to elicit an immune response. Antibodies can either be purified directly, or spleen **cells** can be obtained from the animal. The **cells** are then fused with an immortal **cell** line and screened for antibody secretion. The antibodies can be used to screen clone libraries for **cells** secreting the **antigen** . Those positive clones can then be sequenced (see, for example, Kelly et al., Bio/Technology...

...0072] A purified S. neurona **antigen** bound to a substrate and a ligand specifically reactive with the **antigen** are also contemplated. Such a purified ligand specifically reactive with the **antigen** can be an antibody. The antibody can be a monoclonal antibody obtained by standard methods and as described herein. The monoclonal antibody can be secreted by a hybridoma **cell** line specifically produced for that purpose (Harlow and Lane, 1988). Likewise, nonhuman polyclonal antibodies specifically reactive with the **antigen** are within the scope of the present invention. The polyclonal antibody can also be obtained...of contacting an antibody-containing sample from the subject with a detectable mount of the **antigenic** polypeptide fragment of the present invention and detecting the reaction of the fragment and the...

...sample from the subject with an amount of a purified antibody specifically reactive with the **antigen** as defined herein, and detecting the reaction of the ligand with the **antigen** . It is contemplated that the **antigen** will be on intact **cells** containing the **antigen** , or will be fragments of the **antigen** . As contemplated herein, the antibody includes any ligand which binds the **antigen** , for example, an intact antibody, a fragment of an antibody or another reagent that has reactivity with the **antigen** . The fluid sample of this method can comprise any body fluid which would contain the **antigen** or a **cell** containing the **antigen** , such as blood, plasma, serum, cerebrospinal fluid, saliva, feces and urine. Other possible examples of... immunosorbent assays (ELISA) and immunoblotting can be readily adapted to accomplish the detection of the **antigen** . An ELISA method effective for the detection of the **antigen** can, for example, be as follows: (1) bind the antibody to a substrate; (2) contact the bound antibody with a fluid or tissue sample containing the **antigen** ; (3) contact the above with a secondary antibody bound to a detectable moiety (e.g...

...color change. The above method can be readily modified to detect

antibody as well as **antigen** .

[...

...neurona infection utilizes monoclonal antibodies (MAbs) for detection of antibodies specifically reactive with S. neurona **antigen** . Briefly, sera or other body fluids from the subject is reacted with the **antigen** bound to a substrate (e.g. an ELISA 96-well plate). Excess sera is thoroughly ...

...labeled (enzyme-linked, fluorescent, radioactive, etc.) monoclonal antibody is then reacted with the previously reacted **antigen** serum ... based on monoclonal antibody binding specificity. MAbs can also be used for detection directly in **cells** by IFA...

...detect the presence of S. neurona in a subject. Briefly, latex beads (or red blood **cells**) are coated with the **antigen** and mixed with a sample from the subject, such that antibodies in the tissue or body fluids that are specifically reactive with the **antigen** crosslink with the **antigen** , causing agglutination. The agglutinated **antigen** -antibody complexes form a precipitate, visible with the naked eye or capable of being detected by a spectrophotometer. In a modification of the above test, antibodies specifically reactive with the **antigen** can be bound to the beads and **antigen** in the tissue or body fluid thereby detected...

...typical sandwich assay, the antibody can be bound to a substrate and reacted with the **antigen** Thereafter, a secondary labeled antibody is bound to epitopes ...first antibody and the secondary antibody is detected. Since the present invention provides S. neurona **antigen** for the detection of infectious, S. neurona or previous S. neurona infection other serological methods...

...0079] In the diagnostic methods taught herein, the **antigen** can be bound to a substrate and contacted by a fluid sample such as serum...
...the patient or in a partially purified form. In this manner, antibodies specific for the **antigen** (the primary antibody) will specifically react with the bound **antigen** . Thereafter, a secondary antibody bound to, or labeled with, a detectable moiety can be added...

...antibody or other ligand which is reactive, either specifically with a different epitope of the **antigen** or nonspecific ally with, the ligand or reacted antibody, will be selected for its ability...0081] The **antigen** , e.g., a purified **antigenic** polypeptide fragment encoded by the Sequence Listing of this invention can be used in the construction of a **vaccine** comprising an immunogenic mount of the antigen and a pharmaceutically acceptable carrier. The **vaccine** can be the entire antigen, the antigen on an intact S. **neurona** organism, E. coli or other strain, or an epitope specific to the **antigen** . The **vaccine** can also be potentially cross-reactive with antibodies to other **antigens** . The **vaccine** can then be used in a method of preventing **EPM** or other complications of S. **neurona** infection the **antigen** can be determined using standard procedures. Briefly, various concentrations of a putative specific immunoreactive epitope...

...the vaccine, in which case it can be selected by standard criteria based on the **antigen** used, the mode of administration and the subject (Arnon, R. (Ed.), 1987). Methods of administration can be by oral or sublingual

means, or by injection, depending on the particular **vaccine** used and the subject to whom it is administered...

...0084] It can be appreciated from the above that the **vaccine** can be used as a prophylactic or a therapeutic modality. Thus, the invention provides methods of preventing or treating S. **neurona** infection and the associated diseases by administering the **vaccine** to a subject0085] Nucleic acid **vaccines** against S. **neurona** are also contemplated by the invention. The antigenic agent for use in the **vaccines** of the invention can be any nucleic acid, e.g., as set forth in the...

...to a subject. Suitable nucleic acids include those that encode the native proteins of S. **neurona** , e.g., SnSAG2, SnSAG3 or SnSAG4 protein or a variant or antigenic peptide fragment thereof...

...SEQ ID NO:25 or SEQ ID NO:27. The nucleic acid used as a **vaccine** can be e.g., a naked DNA, or the nucleic acid can be incorporated in...

...0086] The presence of S. **neurona** can also be determined by detecting the presence of a nucleic acid specific for S. neurona or the **antigens** of S. neurona encoded by the nucleic acids set forth herein. The present invention provides...0097] Surface biotinylation of extracellular **merozoites** revealed only two dominant labeled molecules that migrate at about 30 kDa and 16 kDa...

...S. neurona EST database (currently 1800+ sequences) identified an orthologue of the 31-kDa surface **antigen** from **Sarcocystis** muris. The sequence of the S. neurona surface **antigen** gene, designated SnSAG1, is predicted to encode a 276-residue **protein** with an amino-terminal signal peptide and a carboxy-terminal GPI anchor addition. Antiserum raised against recombinant SnSAG1 recognized a 25-kDa **antigen** in western blots of non-reduced S. neurona lysates, consistent with the molecular weight predicted...

...similar to what has been observed in western blot analyses of reduced T. gondii surface **antigens** . Immunofluorescence labeling of SnSAG1 during intracellular growth of S. neurona indicated that the **protein** is expressed throughout schizogony. Interestingly, a filamentous staining pattern was observed in intermediate schizonts that likely reflects localization of the surface **antigen** to previously-described invaginations of the schizont surface membrane...

...0099]S. neurona strain SN3 [Granstrom, 1992 #1600] **merozoites** were propagated by serial passage in bovine turbinate (BT) **cells** and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM sodium pyruvate, Pen/Strep Fungizone (BioWhittaker, Inc.). Extracellular **merozoites** were harvested and purified from disrupted host **cell** monolayers by filtration through 3.0 [small mu, Greek]m membranes, as described previously for...

...0101] Construction and analyses of the cSn.1 S. neurona **merozoite** cDNA library has been described previously [Howe, 2001 #1787]. The library was plaqued for 3 hrs at 42 degree C. on XL 1-Blue MRF' E. coli host **cells** (Stratagene) grown on 150 mm NZY agar plates. When plaques became visible, plates were overlayed0102] **Antigenic** cDNA clones were identified by screening with cerebrospinal fluid (CSF) from a horse that had...

...library [Howe, 1999 #1759] to remove antibodies that were reactive with E. coli and phage **proteins** . After adsorption of potential cross-reactive antibodies, the diluted CSF solution was incubated for 1 ...

...the cSn.1 filters. After washing, filters were incubated for 1 hr with goat anti- **equine** IgG conjugated to horseradish peroxidase (HRP) (Jackson Immunoresearch Labs, Inc.) diluted to 1:10,000...

...clones. Phagemid excision was performed on selected cDNA clones, and plasmids were rescued in SOLR **cells** according to the manufacturer's protocol (Stratagene...0104]S. neurona homologues to previously-characterized coccidian surface **antigens** were identified in the S. neurona clustered EST database (See, e.g., paradb.cis.upenn...

...Information (NCBI) web site (See, e.g., www.ncbi.nlm.nih.gov/) and the Expert **Protein** Analysis System (ExPASy) server of the Swiss Institute of Bioinformatics (See, e.g., www.expasy...to the manufacturer's protocol (Novagen), and monospecific polyclonal antisera were produced against the purified **protein** by immunization of a rabbit and rat (Cocalico Biologicals, Inc...mercaptoethanol, and the lysates were separated in 10% or 12% polyacrylamide gels [Laemmli, 1970 #393]. **Proteins** were transferred to nitrocellulose membranes by semidry electrophoretic transfer in Tris-glycine buffer (pH 8...

...0109] Biotinylation of Surface **Proteins** and Precipitation with Immobilized Streptavidin...

...0110] Approximately 3X10[sup]7 freshly harvested **merozoites** were resuspended in 1 ml cold PBS (pH 7.8). Sulfo-N-hydroxy-succinimide-biotin ...and the sample was centrifuged at 16,000Xg to remove the insoluble fraction. The soluble **proteins** were incubated with UltraLink immobilized streptavidin (Pierce), and the precipitated biotin-labeled **protein** fraction was analyzed by western blotting, as described above. Immunofluorescent labeling of extracellular and intracellular...

...detection of SnSAG1 on extracellular parasites and in trails deposited by gliding parasites, freshly lysed **merozoites** were suspended in fresh RPMI 1640 and incubated on poly-L-lysine-coated slides for...

...2.5% formalin-PBS containing 0.01% glutaraldehyde. For detection of SnSAG1 on intracellular parasites, **merozoites** were inoculated onto BT **cells** grown on LabTek chamber slides (Nunc). At 24 hr, 48 hr, or 72 hr post-inoculation, the **cells** were fixed in 2.5% formalin-PBS/0.01% glutaraldehyde and permeablized with 0.20114] To obtain a preliminary identification of the parasite **protein** encoded by the selected cDNAs, the SnAgI.9 clone was used to affinity purify antibodies that bind the **antigen** expressed by this clone, and the eluted antibodies were used to probe a western blot of S. neurona **merozoite** lysate. As shown in FIG. 1, the purified antibodies reacted with an approximately 31-kDa **antigen** in reduced S. neurona lysate. Furthermore, the **antigen** revealed by the phage-purified antibodies comigrated with a **protein** that is recognized by **equine** or rabbit antisera against S. neurona as the major immunodominant **antigen** of this parasite (FIG. 1, lanes 2 and 3). This result implies that the 22...

...during the library screen and represented by SnAgI.8 and SnAgI.9 encode the immunodominant **antigen** of S. neurona...

...of 1493 nucleotides, with an open reading frame (ORF) that encodes a 276 amino acid **protein** . Sequence analysis of SnAgI.9 indicated that this clone was virtually identical to SnAgI.8...

...160 nucleotides longer due to an alternative polyadenylation site. A hydrophobicity plot of the encoded **protein** showed hydrophobic domains at both termini, which correspond to a predicted signal peptide at the... 143. Removal of the N-terminal and C-terminal signal sequences results in a mature **protein** of 242 amino acids that has a predicted molecular weight of 24.2 kDa before...

...the query. These searches revealed a statistically significant similarity to the 31 kDa major surface **antigen** of **Sarcocystis** muris [Eschenbacher, 1992 #1767] and a less significant but recognizable similarity to several SAG2-related surface **antigens** from T. gondii [Lekutis, 2000 #2049]. (FIG. 2). In conjunction with the western blot analysis...

...the gene represented by the SnAgI.8 and SnAgI.9 cDNAs encodes an immunodominant surface **antigen** of S. neurona; consequently, we tentatively designated this **protein** SnSAG1, following the genetic nomenclature that is utilized for the related apicomplexan parasites T. gondii...a fashion similar to that set forth above for SnGAG1. These novel nucleotide sequences and **protein** sequences of **Sarcocystis neurona** can be utilized in the production of **vaccines** and/or **antigen** /antibody kits for prevention and diagnosis of **Sarcocystis neurona** infection. One preferred embodiment of the invention is a **vaccine** comprised of an alpha virus expression vector and nucleic acid selected from the nucleic acid...

...Identification ofS. **neurona** Surface **Antigens** and Expression as Recombinant **Proteins**

[...

...0118] Analysis of the S. neurona EST database revealed four paralogous **proteins** that are homologous to the SAG and SRS surface **antigens** of Toxoplasma gondii. Each S. neurona gene was predicted to encode a **protein** that possessed an amino-terminal signal peptide and a carboxyl-terminal glycolipid anchor site, consistent with the **proteins** being surface **antigens** . Because of their similarity to Toxoplasma SAGs and their probable surface display on **merozoites** , the four S. neurona **proteins** were designated SnSAG1, SnSAG2, SnSAG3, and SnSAG4. The four putative surface **antigens** were each expressed as a recombinant **protein** in E. coli, and these were used to immunize rabbits and rats for monospecific polyclonal...

...forms of native SnSAG1 and SnSAG4 are predicted to be approximately 24 kDa, but these **antigens** co-migrated at approximately 30-32 kDa and correspond to the immunodominant **antigen** Sn30 that has been described previously (See, FIG. 3) (Granstrom et al., 1993; Liang et al., 1998). SnSAG1 has also been identified by others as a major surface **antigen** matching the immunodominant Sn30 band (Ellison et al., 2002), but it is apparent that SnSAG4...

...weight. The mature form of SnSAG2 is predicted to be about 12 kDa, but this **antigen** migrated at approximately 18-19 kDa and corresponds to the previously described immunodominant Sn16 **antigen** (See, FIG. 3) (Granstrom et al., 1993; Liang et al., 1998). Mature SnSAG3 is predicted ...

...SnSAGs under reducing conditions is a characteristic that has been observed previously for the surface **antigens** of both T. gondii (Burg et al., 1988; Cesbron-Delauw et al., 1994) and N...between antibody recognition of recombinant SnSAG1 (rSnSAG1) and standard western blot analysis of complete parasite **antigen** (i.e., S. neurona **merozoite** lysate). Similar results were obtained with rSnSAG2, rSnSAG3, and rSnSAG4. These data demonstrate the utility...

...Enzyme-Linked Immunosorbent Assays (ELISAs) Based on RecombinantS. neurona Surface **Antigens** (rSnSAGs...

...rSnSAGs expressed in E. coli have been shown in western blots to be recognized by **equine** antibodies; consequently, these recombinant **antigens** can be utilized as the key reagents for developing ELISAs based on single S. neurona **antigens** . Given the teachings set forth herein and utilizing methods known in the art, an ELISA...0121] To produce highly purified recombinant forms of the SnSAGs, the genes for each **antigen** have been cloned into the pET22b expression plasmid from Novagen (Madison, Wis.). This plasmid vector...

...ion affinity columns and allows for the efficient one-step purification of the expressed recombinant **protein** . Plasmid constructs were transformed into BL21 (DE3) host **cells** (CodonPlus, Stratagene, Inc.), and expression of recombinant **protein** was induced by addition of IPTG. Bacterial clones that reliably expressed the recombinant SnSAGs were selected and cyropreserved for future study. The recombinant S. neurona surface **antigens** have been designated rSnSAG1, rSnSAG2, rSnSAG3, and rSnSAG4. When recombinant **protein** is needed for use in the ELISAs, the appropriate bacterial clone can be grown to logarithmic phase in LB medium, and **protein** expression can be induced by addition of IPTG to the culture. The recombinant **protein** can be extracted from inclusion bodies with 6 M urea and purified from the host **cell** lysate by Ni++-column chromatography according to the manufacturer's protocol (His-Bind resin and buffers, Novagen). To remove the urea, purified recombinant **proteins** can be dialyzed into 350 mM NaCl, 10% glycerol, 50 mM NaH2PO4, 5 mM MgCl2 and stored at -20C until used. If necessary, recombinant **proteins** can be concentrated by centrifugal ultrafiltration in Centricon-10 columns (Amicon...times with PBS/0.1% Tween 20 and incubated with horseradish peroxidase (HRP)-conjugated anti- **equine** immunoglobulin secondary antibody (Jackson Immunoresearch Labs, Inc.). The wells can again be washed with PBS...0127] Bentz, B. G., D. E. Granstrom, and S. Stamper. 1997. Seroprevalence of antibodies to **Sarcocystis** neurona in horses residing in a county of southeastern Pennsylvania [see comments]. J Am Vet...

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Exemplary or Independent Claim(s):
...7. A composition comprising an isolated nucleic acid capable of encoding an **antigenic protein** derived from **Sarcocystis** neurona or a unique **antigenic** polypeptide fragment thereof comprised of at least a portion of a nucleotide sequence selected from purified **antigenic** polypeptide comprised of at least a portion of an amino acid sequence selected from the...

...13. A composition comprising a purified antibody that is specifically reactive with a a **antigenic** polypeptide comprised of at least a portion of an amino acid sequence selected from the...

...17. A method for detecting **Sarcocystis** neurona in a biological sample comprising detecting the presence of: (a) a nucleic acid comprising ...23; SEQ ID NO: 25; SEQ ID NO: 27; SEQ ID NO: 29; (b) an **antigenic** polypeptide comprised of at least a portion of an amino acid sequence selected from the...

...28; and SEQ ID NO: 30; and (c) an antibody that specifically binds to a **antigenic** polypeptide comprised of at least a portion of an amino acid sequence selected from the18. A composition for stimulating an immune response against **Sarcocystis** neurona when administered to an animal, the composition comprising an immunogenic amount of: (a) an ...

...agent that can specifically stimulate an immune response against at least a portion of a **protein** or polypeptide wherein the **protein** or polypeptide is selected from the group set forth in the Sequence Listing as SEQ...

Non-exemplary or Dependent Claim(s):
...wherein the isolated nucleic acid is capable of selectively hybridizing with a nucleic acid from **Sarcocystis** neurona...14The composition of claim 13, wherein the antibody is specifically reactive with **Sarcocystis** neurona...

10/3,KWIC/17 (Item 12 from file: 654)
DIALOG(R)File 654:US Pat.Full.

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Detection of sarcocystis **neurona**
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Detection of sarcocystis **neurona**

Abstract:

A gene encoding a 29 kilodalton **protein** found on the surface of **merozoite** stage S. **neurona** has been cloned and sequenced. The **protein** encoded by this gene, termed SnSAG-1, is an immunodominant **antigen** recognized on **protein** blots. Methods for using nucleic acids and polypeptides relating to SnSAG-1 in diagnostic tests and **vaccine** development are disclosed...

Summary of the Invention:

...microbiology and veterinary medicine. More particularly, the invention concerns compositions and methods relating to detecting **Sarcocystis** neurona...

...0004] **Equine** Protozoal Myeloencephalitis (**EPM**) is a common cause of neurologic disease in New World horses. It is caused by a parasite termed **Sarcocystis** neurona (S. neurona), an obligatory intracellular apicomplexan parasite whose multi-phase life cycle is completed...horse has been infected, S. neurona can travel to the brain and spinal cord, where **merozoite** stages of this parasite replicate and cause pathology ...

...0005] Horses with **EPM** typically present with lameness, but may alternatively or additionally present with symptoms characteristic of primary...

...inhabit any area of the central nervous system (CNS) of the horse, symptoms associated with **EPM** can vary widely. The degree of infection can range from subtle to severe and can involve the brain and/or the spinal cord. **EPM** is usually progressive...

...0006] Presently, a definitive diagnosis of **EPM** is made by post-mortem examination, where S. neurona organisms are identified in histological lesions...

...or when cultured from the lesion establishes the diagnosis. Heretofore, pre-mortem methods for diagnosing **EPM** were based on assays using whole **merozoites** , and not a purified **protein** , to probe for the presence of anti-S. neurona antibodies (as an indication of infection) in the horses. The use of such whole **merozoites** results in significant cross-reaction with non-S. neurona specific antibodies (e.g., those against other **Sarcocystis** species). This cross-reactivity obscures interpretation of results using whole **merozoite** -based assays...

...0007] The invention relates to the discovery and characterization of a 29 kilodalton (kDa) **protein** found on the surface of **merozoite** stage S. neurona. This **antigen** , termed SnSAG-1 or SnSMA1, is an immunodominant **antigen** recognized on **protein** blots. Using purified or recombinant SnSAG-1 (i.e., rSnSAG-1) **antigen** , accurate assays for diagnosing **EPM** in horse pre-mortem have been developed. These assays involve identifying a marker indicative of the presence of the 29 kDa **antigen** or an antibody to this **antigen** in a sample to be tested. Thus, because a single purified **antigen** or marker is utilized in such assays, the cross-reactivity problems associated with whole-merozite SnSAG-1 **antigen** has been cloned from a gene library prepared from an isolate of S. **neurona** . The original clone was identified in a collection of random sequence tags prepared to characterize...

...This sequence or the clone itself can be used to gusto prepare the SnSAG-1 **antigen** in a recombinant or other synthetic form for use in diagnostic tests and **vaccine** development...

...0009] Accordingly, the invention features a composition for detecting the presence of S. **neurona** in a biological sample. In one variation, the composition includes a SnSAG-1 marker that is a purified nucleic acid including a nucleotide sequence that encodes a **protein** that shares at least 50% or at least 90% sequence identity with SEQ ID NO:1. In this variation, the nucleotide sequence can also encode the **protein** of SEQ ID NO:1. For example, the nucleotide sequence can be SEQ ID NO...0011] In a third variation of the composition, the SnSAG-1 marker is an isolated **protein** including a polypeptide that shares at least 50%, 70%, 90%, or 95% sequence identity with...

...include the entire amino acid sequence of SEQ ID NO:1. In this composition, the **protein** can be a fusion **protein** or a recombinant **protein** .

[...0013] In another aspect, the invention features a method for detecting **Sarcocystis** neurona in a biological sample. This method includes the steps of: (a) providing the biological...

...SnSAG-1 marker that is a nucleic acid including a nucleotide sequence that encodes a **protein** that shares at least 50% sequence identity with SEQ ID NO:1; a polynucleotide that...

...ID NO:3, wherein the polynucleotide can be at least 30 nucleotides in length; a **protein** including a polypeptide that shares at least 50% sequence identity with a fragment of the...

...of the SnSAG-1 marker in the biological sample indicates that the biological sample contains **Sarcocystis** neurona a nucleic acid including a nucleotide sequence that encodes a **protein** that shares at least 50% sequence identity with SEQ ID NO:1, the nucleotide sequence...

...0015] In the variation of this method where the SnSAG-1 marker is a **protein** including a polypeptide that shares at least 50% sequence identity with a fragment of the...the sample to a molecule that specifically binds to an antibody that specifically binds a **protein** consisting of the amino acid sequence of SEQ ID NO:1. In the latter, the ...a SnSAG-1 marker includes contacting the sample with a molecule that specifically binds a **protein** consisting of the amino acid sequence of

SEQ ID NO:1. The molecule can be...

...0020] The invention also features a composition for stimulating an immune response against **Sarcocystis** neurona when administered to an animal. The composition includes (a) an isolated agent that can specifically stimulate an immune response against a **protein** consisting of the amino acid sequence of SEQ ID NO:1 when administered to an...

...salt; an oil-in-water emulsion; a composition including saponin; a composition including a bacterial **protein** ; or a cytokine...

...0021] The agent that can stimulate an immune response against **Sarcocystis** neurona when administered to an animal can include a nucleic acid that can be a first polynucleotide including a nucleotide sequence that encodes a **protein** that shares at least 50% sequence identity with SEQ ID NO:1; or a second...

...By the term "gene" is meant a nucleic acid molecule that codes for a particular **protein** , or in certain cases, a functional or structural RNA molecule. For example, the SnSAG-1 gene encodes the SnSAG-1 **protein** ... acid molecule is one that is substantially separated from other nucleic acid sequences in a **cell** or organism in which the nucleic acid naturally occurs (e.g., 30, 40, 50, 60...0026] As used herein, the terms " **protein** " and "polypeptide" are used synonymously to mean any peptide-linked chain of amino acids, regardless...

...is one that has been substantially separated or isolated away from other polypeptides in a **cell** , organism, or mixture in which the polypeptide occurs (e.g., 30, 40, 50, 60, 70...

...included on a substrate (e.g., a polyacrylamide gel) with other purified polypeptides from the **cell** or organism in which the polypeptide occurs ...

...0027] By the terms "SnSAG-1 **protein** " or "SnSAG-1 polypeptide" are meant an expression product of a SnSAG-1 nucleic acid (e.g., one consisting of SEQ ID NO:3), or a **protein** that shares at least 50% (but preferably 55, 60, 65, 70, 75, 80, 85, 90...20, 30, 40, 50, 75, 100 or more amino acids of a native SnSAG-1 **protein** ...a "SnSAG-1 marker" is any molecule whose presence in a sample (e.g., a **cell**) indicates that a SnSAG-1 gene or **protein** is present in the sample or subject from which the sample was derived. SnSAG-1 markers include SnSAG-1 nucleic acids, SnSAG-1 **proteins** , and antibodies that specifically bind SnSAG-I **proteins** . "Expressing a SnSAG-1 gene" or like phrases mean that a sample contains a transcription...

...e., "mRNA") of a SnSAG-1 gene or a translation product of a SnSAG-1 **protein** -encoding nucleic acid (e.g., a SnSAG-1 **protein**). A **cell** expresses a SnSAG-1 gene when it contains a detectable level of a SnSAG-1 nucleic acid or a SnSAG-1 **protein** ...or receptor, refers to a binding reaction which is determinative of the presence of the **protein** or polypeptide or receptor in a heterogeneous population of **proteins** and other biologics. Thus, under designated conditions (e.g. immunoassay conditions in the case of...

...binds to an SnSAG-1polypeptide) and does not bind in a significant amount to other **proteins** present in the sample or to other **proteins**

. to which the ligand or antibody may come in contact in an organism... response" is meant eliciting or increasing the activation of a lymphocyte (e.g., a B **cell** or T **cell**) or other immune system component. The stimulation of an immune response against a specific **antigen** can be measured as an increase in antibody titer against that **antigen** or the activation of one or more lymphocytes having a surface receptor specific for the **antigen** . Activation of lymphocytes can be determined by conventional assays, e.g., the induction of mitosis, secretion of cytokines, modulation of **cell** surface molecule expression, secretion of immunoglobulin (B **cells**), and increased killing of target **cells** (cytotoxic T **cells**).

[0037] A gene encoding a S. neurona surface **antigen** has been cloned and sequenced. The **antigen** encoded by the gene has been characterized. Rabbit anti-S. neurona polyclonal antibody was used to immunoprecipitate and concentrate **proteins** of an isolate of S. neurona for detection of antibodies in body fluids of clinically...

...horses. The serum and cerebral spinal fluid (CSF) of diseased animals was used to identify **antigens** important in natural infections. Techniques were developed to separate parasites from host **cells** facilitating production a cDNA expression library. The library was screened with both polyclonal rabbit anti-S. neurona and mass culture supernatant from hybridoma **cells** produced from mice immunized with the S. neurona isolate. A cone was also identified in...

...a probe to select the full length copy of the gene encoding a major surface **antigen** , SnSAG-1, of S. neurona. The sequence data from the full length gene was used...BamH1 site of the expression vector pET14b which allows expression of a His-tagged recombinant **protein** (i.e., His-tagged rSnSAG-1). This recombinant **protein** migrated slightly larger on SDS-PAGE than the native **antigen** .

[...

...0039] A monoclonal antibody that specifically binds an epitope of the 29 kDa **protein** (corresponding to SnSAG-1) from cultured S. neurona **merozoites** was used to verify the presence of the epitope on the recombinant **protein** . The recombinant **protein** was purified and used to produce a monospecific polyclonal antibody in mice and goats. The anti-SnSAG-1 antisera was used to characterize the 29 kDa **antigen** of cultured S. neurona **merozoites** as a surface **protein** .

[...be performed, for example, on commercial automated oligonucleotide synthesizers. Immunological methods (e.g., preparation of **antigen** -specific antibodies, immunoprecipitation, and immunoblotting) are described, e.g., in Current Protocols in Immunology, ed...

...SEQ ID NO:2. The region of this nucleic acid encoding the native SnSAG-1 **protein** (see SEQ ID NO:3) is found in positions 73-903 (SEQ ID NO:3... or non-coding (anti-sense) strand. The coding sequence which encodes the native SnSAG-1 **protein** may be identical to the nucleotide sequence shown herein as SEQ ID NO:3. It...gene such as those that encode fragments, analogs and derivatives of a native SnSAG-1 **protein** . Such variants may be, e.g., a naturally occurring allelic variant of the native SnSAG...

...0046] Variant SnSAG-1 **proteins** displaying substantial changes in structure can be generated by making nucleotide substitutions that cause less...

...an amino acid side chain. Nucleotide substitutions generally expected to produce the greatest changes in **protein** properties are those that cause non-conservative changes in codons. Examples of codon changes that are likely to cause major changes in **protein** structure are those that cause substitution of (a) a hydrophilic residue, e.g., serine or...the native SnSAG-1 gene, and encode polypeptides having structural similarity to native SnSAG-1 **protein** . Homologs of the native SnSAG-1 gene within the invention are nucleic acids isolated from...

...the native SnSAG-1 gene, and encode polypeptides having structural similarity to native SnSAG-1 **protein** . Public and/or proprietary nucleic acid databases can be searched to identify ...the native SnSAG-1 gene, and encode polypeptides having structural similarity to native SnSAG-1 **protein** (e.g., those that cross react with antibodies that specifically bind the native SnMS1 **protein**). Examples of non-naturally occurring SnSAG-1 gene variants are those that encode a fragment of a SnSAG-1 **protein** , those that hybridize to the native SnSAG-1 gene or a complement of to the...

...complement of the native SnSAG-1 gene, and those that encode an SnSAG-1 fusion **protein**

Description of the Invention:
...Murphy A. J. and Mansfield, L. S., 1999 Journal of Parasitology 85(5):979-981. **Sarcocystis** neurona sporocyst isolates were selected from among those identified using DNA marker analysis. Dame J...

...of 100 [small mu, Greek]l PBS. 2) ~100 sporocysts were resuspended in PBS containing **proteinase** K (1 mg/ml) and 1% SDS and incubated for 10 min. at 37 degree...a 25 cm[sup]2 flask with a freshly trypsinized, 60% confluent monolayer of BM **cells** in Dulbecco's medium containing 10% horse serum, 100 units/ml penicillin, 100 units/ml...
...Ionophore A23187-Stimulated **Merozoite** Release and Purification...to a final concentration of 1 mg/ml and stored at -20 degree C. Infected **cell** monolayers (see example 1) at 12 days post infection were washed three times with PBS...

...at 37 degree C. for 40 min. in 5% CO[sub]2, 95% air. Free **merozoites** were collected by centrifugation and washed in PBS as above. Parasites released by this method were examined by density gradient centrifugation, but further separation from host **cell** debris by density gradient centrifugation was not routinely necessary. For further purification, parasites recovered from the supernatant were isolated from host **cell** debris on a discontinuous buoyant density gradient using Iodixanol (Optiprep) in PBS or HBSS. **Merozoites** were suspended gently in 1.0 ml PBS and were layered onto a preformed, three...

...at each interface were collected and examined microscopically. The fractions(s) containing signiicant amounts of **merozoites** were collected for use in the culture medium was enhanced 90 fold by incubation of infected host **cells** for 40 min in 1 [small mu, Greek]M A23187 prior to collecting the culture supernatant. Parasitized host **cells** that released **merozoites** in response to ionophore treatment were BM, BM0617,

HL, BT, and ED **cells** . Infected GT, HFF, CHO, BHK, and primary EM **cells** were refractory for the release of parasites under the same conditions. The release of parasites...

...observed free in the culture supernatant. Although difficult to accurately determine the percentage of individual **merozoites** released by this treatment, it was a large proportion. No mature schizonts were visible in a microscopic examination of treated cultures. Selective disruption of the parasitized-host- **cell** membrane was seen in electron micrographs. Initially, the host **cell** increased in size, became vacuolated, and had small breaks in the membrane. As the plasma...

...membrane bound vacuoles or vacuoles with peripheral ribosomes were released into the media. The host **cell** became long and cytoplasmic volume decreased. Parasites were observed to move beginning at 10 minutes and continuing until their release at forty minutes. The **merozoites** undulated hyperactively in this media, but with the removal of the ionophore by addition of culture media, released parasites and the host **cells** recovered a normal appearance and activity. Ionophore-treated parasites remained animated and readily infected new **cells** when incubated onto a fresh monolayer. The difference noted in electron micrographs of ionophore treated...

...that they appeared to have more prominent micronemes than untreated parasites. The separation of parasites **proteins** after they are treated with ionophore was observed, however coomassie blue staining is not sufficient to distinguish parasite **antigens** from those of the host **cells** .

[...

...0095] Parasites released from the host **cell** monolayer by A23187 were single, hyperactive, and entered **cells** readily. When A23187-treated parasites were used as the inoculum, the infected host **cells** harbored an abundance of mature schizonts in 3 to 5 days. The extracellular parasites remaining after washing off the ionophore did not re-invade ionophore-treated host **cells** , but increased in size while the few **merozoites** that remained in host **cells** formed mature schizonts in five days continuing the infection. Ten days after ionophore treatment, the...

...thirty days, ionophore treatment again elicited parasite release. During this second ionophore treatment, many host **cells** were released into the supernatant0096] **Sarcocystis** neurona was grown in 11 **cell** lines to determine growth rate and response to ionophore treatment of the monolayer. **Sarcocystis** neurona **merozoites** replicated in two different bovine monocyte lines [BM, laboratory stock culture and BM 0617 (American Type Culture Collection, Rockville, Md., USA) CRL 0617], bovine turbinate **cells** (BT **cells** , ATTC CRL 1390), human lung **cells** (HL **cells** , ATTC CCL 201-8Lu), human foreskin fibroblasts (HFF, ATTC CRL 2450), Chinese hamster ovary **cells** (CHO **cells** , ATTC CCL 61), bovine kidney (MDBK **cells** , ATTC CCL 22), goat tumor **cells** (GT **cells** , a gift of Dr. Jack Gaskin), **equine** dermal **cells** (ED **cells** , ATTC CRL 6288), and **equine** monocytes (EM, primary culture from peripheral blood). Parasite growth in each of these host **cell** lines was observed over a 30 day period starting from an inoculum of 2000 parasites collected from the culture supernatant of BM **cells** .

[...

...growth with rosette formation at five days post infection when sub-cultured from BM 0617 **cells** . Formation of rosettes was first observed 3 days post infection with release and re-invasion of new **cells** occurring at five days post infection. The efficiency of infection was increased when freshly trypsinized host **cells** were placed in sufficient numbers in the culture flask to establish a 60% confluent monolayer immediately before **merozoites** were added. This increase in numbers of **merozoites** entering host **cells** improved the yield of parasites and shortened by two weeks the length of time required for culture prior to harvesting the **merozoites** . Infection of BT **cells** by **merozoites** was increased by 50% using scraped **cells** from thirty day post infection as inoculum rather that the supernatant from the same cultures...

...Examination of Parasites Found Free in Culture vs. Inside Host **Cells**

[...

...Studies of the replication of the parasite were performed as follows. Approximately 220 S. neurona **merozoites** recovered from a culture supernatant were added to 5000 Human Lung (HL) **cells** seeded and growing on Thermonax coverslips in 24 well plates to evaluate the growth and...

...days, the 2 ml supernate was removed and evaluated by cytospin and the number of **merozoites** present counted. The corresponding coverslip was ...0099] Cultures of parasites replicating in HL **cells** were examined to follow the number of parasites free in the culture supernatant as compared with those found inside host **cells** during normal parasite growth. The majority of the parasites were released at 21 days in...

...0100] Lung **cells** at 60% confluence were seeded at a density of 0.1 **merozoites** per host **cell** and parasite growth was monitored microscopically for 30 days. Free parasites were not observed until...

...to less than 10%. Duplicate 75 cm flasks were grown for 12 days in HL **cells** and treated with HBSS or HBSS with 1 [small mu, Greek]M A23187. Free parasites...described by Tanhauser S., et al. 1999 Journal of Parasitology 85(2):221-228. Washed **merozoites** were pelleted by centrifugation for five minutes at 16,000 g in a microcentrifuge. The...

...MgCl[sub]2, 1% Triton X-100, 1% Tween-20, 1 [small mu, Greek]M **Proteinase** K), incubated two hours at 56 degree C., and then boiled for two min. to inactivate the **Proteinase** K. The tube was centrifuged to remove particulate matter and the supernate was used directly...2D Electrophoresis and Immunoprecipitation ofS. neurona **Antigens**

Exemplary or Independent Claim(s):

...group consisting of: (A) a purified nucleic acid comprising a nucleotide sequence that encodes a **protein** that shares at least 50% sequence identity with SEQ ID NO:1; (B) a purified...

...NO:3, wherein the polynucleotide is at least 30 nucleotides in length; (C) an isolated **protein** comprising a polypeptide that shares at least 50% sequence identity with a fragment of the...

...24. A method for detecting **Sarcocystis** neurona in a biological sample, the method comprising the steps of: (a) providing the

biological...

...from the group consisting of: a nucleic acid comprising a nucleotide sequence that encodes a **protein** that shares at least 50% sequence identity with SEQ ID NO:1; a polynucleotide that nucleotides in length; a **protein** comprising a polypeptide that shares at least 50% sequence identity with a fragment of the...

...of the SnSAG-1 marker in the biological sample indicates that the biological sample contains **Sarcocystis** neurona...

...50. A composition for stimulating an immune response against **Sarcocystis** neurona when administered to an animal, the composition comprising: (a) an isolated agent that can specifically stimulate an immune response against a **protein** consisting of the amino acid sequence of SEQ ID NO:1 when administered to an...

Non-exemplary or Dependent Claim(s):
...the SnSAG-1 marker is the nucleic acid comprising a nucleotide sequence that encodes a **protein** that shares at least 50% sequence identity with SEQ ID NO:1...

...3. The composition of claim 2, wherein the nucleotide sequence encodes a **protein** that shares at least 90% sequence identity with SEQ ID NO:1...

...4. The composition of claim 2, wherein the nucleotide sequence encodes the **protein** of SEQ ID NO:1...10. The composition of claim 1, wherein the SnSAG-1 marker is the isolated **protein** comprising a polypeptide ...19. The composition of claim 10, wherein the **protein** is a fusion **protein** .
...

...20. The composition of claim 10, wherein the **protein** is a recombinant **protein** .
...the SnSAG-1 marker is the nucleic acid comprising a nucleotide sequence that encodes a **protein** that shares at least 50% sequence identity with SEQ ID NO:1...28. The method of claim 24, wherein the SnSAG-1 marker is the **protein** comprising a polypeptide that shares at least 50% sequence identity with a fragment of the...the sample to a molecule that specifically binds to an antibody that specifically binds a **protein** consisting of the amino acid sequence of SEQ ID NO:1...a SnSAG-1 marker comprises contacting the sample with a molecule that specifically binds a **protein** consisting of the amino acid sequence of SEQ ID NO:1...salt; an oil-in-water emulsion; a composition comprising saponin; a composition comprising a bacterial **protein** ; and a cytokine...

...The composition of claim 50, wherein the agent that can stimulate an immune response against **Sarcocystis** neurona when administered to an animal comprises a nucleic acid selected from the group consisting of a first polynucleotide comprising a nucleotide sequence that encodes a **protein** that shares at least 50% sequence identity with SEQ ID NO:1; and a second...

10/3,KWIC/20 (Item 15 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4745296
Derwent Accession: 1998-110331
Utility
REASSIGNED
C/ **Treatment of** equine **protozoal myeloencephalitis** ; CAUSED **BY INFECTION BY THE PROTOZOAN PARASITE** SARCOCYSTIS **NEURONA (RECENTLY REFERRED TO AS** SARCOCYSTIS **FALCATULA)**
Inventor: Russell, Meri Charm, Des Moines, IA
Fenger, Clara K., Lexington, KY
Assignee: IDEXX Pharmaceuticals, Inc.(02), Greensboro, NC
Blue Ridge Pharmaceuticals Inc (Code: 55591)
Examiner: Weddington, Kevin E. (Art Unit: 164)
Law Firm: Foley & Lardner

	Publication Number	Kind	Date	Application Number	Filing Date
Main Patent	US 6448252	A	20020910	US 2000685943	20001010
Continuation	US 6255308	A		US 9869956	19980430
Continuation	US 5747476	A		US 96683507	19960717

Fulltext Word Count: 4189

Treatment of equine **protozoal myeloencephalitis**

Abstract:
The present invention relates to compositions and methods for treating **equines** , such as horses, afflicted with **equine** protozoal myeloencephalitis or **EPM** . The therapeutic compositions comprise a combination of pyrimethamine and a sulfonamide, preferably, sulfadiazine, in the...

Summary of the Invention:
...The present invention relates to compositions and methods for treating **equines** , such as horses, afflicted with **equine** , protozoal myeloencephalitis of **EPM** . **EPM** is a debilitation neurologic disease of **equines** which can affect the brain, the brain stem, spinal cord, or any combination of these three areas of the **equine** 's central nervous system. **EPM** is caused by infection by the protozoan parasite **Sarcocystis neurona** (recently referred to as **Sarcocystis** falcatula). There is no **vaccine** or approved animal drug product available for effectively treating this disease in horses...

...Although the symptoms and effects of **EPM** have been recognized since the 1970's, it was not until 1991 that the protozoan parasite that causes **EPM** was cultured from a horse and given the name **Sarcocyscis** neurona. The horse is an aberrant, dead-end host, as infectious forms of the parasite... **EPM** occurs in much of North America. Serologic surveys conducted in central Kentucky one county in...

...environment was associated with a decrease in the numbers of horses exposed to the parasite. **EPM** appears to have a sporadic distribution, although outbreaks have occurred on farms in Kentucky, OhioA horse of any age, breed, or sex may be affected by **EPM** . The disease has occurred in a horse of two months of age, as well as...

...its thirties. In fact, any horse demonstrating neurologic abnormalities should be considered a candidate for **EPM** affliction...

...head tilt with asymmetry of the face (e.g., eyelid, ear, or lip). A severely **EPM** -affected horse may become recumbent and unable to rise. Lameness not traceable to orthopedic disease or any combination of the above signs may occur with **EPM** . Other unusual signs may also occur... Diagnosis of **EPM** is based on clinical signs and on testing of the horse's cerebrospinal fluid (CSF). Originally, the diagnosis was based on the presence of antibodies to **Sarcocystis** neurona in serum, though it is now known that a positive serum test cannot be...

...Currently available treatment of horses with **EPM** is expensive and typically requires a duration of at least ninety (90) days. In some... Adverse effects of therapy may include anemia, abortion, diarrhea and low white blood **cell** counts. Both medications for treatment of **EPM** inhibit folic acid metabolism. Unlike horses, however, the protozoan is unable to utilize pre-formed...16.7 mg/kg) and trimethoprim (3.3 mg/kg), to treat horses suffering from **EPM** . See, Welsch, B. B. , in The Compendium North American Edition, **Equine** , Morris, D. D. (Ed.) (1991) pp. 1599-1602...of past and on-going effort, there remains an unfulfilled need for a treatment for **EPM** -afflicted **equines** , particularly horses, which is not only effective but is also convenient to administer to maximize...

...and reduce the emergence of resistant strains. In particular, prior compositions for the treatment of **EPM** involve three-component mixtures, including pyrimethamine, sulfadiazine and trimethoprim. Moreover, where prior compositions contained pyrimethamine...

...malaria only and hampering their usefulness in other pathological conditions, like protozoan-mediated diseases, especially **EPM** . The fact is that there is currently no approved drug or drug combination for the treatment of **EPM** ...Quite surprisingly, it has now been discovered that an effective, convenient method of treating **EPM** is realized by the administration to an **equine** suspected of being afflicted with **EPM** of therapeutic amounts of pyrimethamine and a sulfonamide, preferably sulfadiazine. The relative weight ratio of...

...the weight amount of sulfadiazine present. Preferably, the therapeutic compositions used for the treatment of **EPM** are substantially free of trimethoprim, most preferably having no trimethoprim at all. Similarly, the methods...

...not rely on the presence of significant amounts of trimethoprim in effecting successful treatment of **EPM** , using substantially the pyrimethamine and a sulfonamide as the principal active ingredients against the pathologic agent, namely, the organism **Sarcocystis** neurona in **EPM** . Hence, the methods of the present invention do not include the co-administration of known...

...In a preferred embodiment of the invention, the afflicted **equine** , e.g., a horse, is given a daily dose of pyrimethamine, which is equivalent to about 0.8-1.2 mg per kg of **equine** , most preferably about 1.0 mg per kg. The subject is also given, concurrently for...

...day of a sulfonamide, which is equivalent to about 15-30 mg per kg of **equine** , most preferably about 20 mg per kg. Once daily administration of

the active ingredients, say...

...It should be apparent that an object of the present invention is the treatment of **equine** protozoal myeloencephalitis or **EPM** by providing a veterinary composition comprising pyrimethamine and a sulfonamide, provided that the composition ...preferably, the veterinary composition of the present invention (or the instant method of treatment of **EPM**) is substantially free of trimethoprim...a sulfonamide, such as sulfadiazine, designed to overcome the shortcomings of currently available treatments of **EPM** and to provide a more effective drug combination for horses and other **equines** infected with an organism of the genus **Sarcocystis** . As previously mentioned, pyrimethamine may be given in a preferred dose of about 1 mg/kg **equine** with a sulfonamide in a dose of about 15 to 30 mg/kg **equine** , preferably 20 mg/kg...

...described, below) daily on an empty stomach will provide adequate dosing for the treatment of **EPM** that neither pyrimethamine nor sulfadiazine can treat alone. Since **EPM** is a protozoal infection of the central nervous system, the appropriate drug combination must penetrate... composition may be prepared in unit dosage form depending upon the minimum size of the **equine** . Such unit dosage forms comprise a relative weight ratio of pyrimethamine to sulfonamide in a...

...The present invention has been found to successfully inhibit the growth of the organism **Sarcocystis** neurona in **equines** , such as mules, ponies and horses. It has been observed that the preferred sulfonamide, sulfadiazine...invention to employ compositions utilizing one or more sulfonamides and/or pyrimidine derivative in treating **EPM** . Examples of other suitable pyrimidine derivatives include, but are not limited to, 2,4-diamino...

Description of the Invention:

...Veterinary compositions effective for the general treatment of **EPM** are provided, below, in the form of an oral suspension. The amounts of each component...

...mentioned above, a useful dosage, e.g., for a 1,000 pound horse infected with **Sarcocystis** neurona (as evidenced by the presence of the protozoan in a sample from the subject...the subject may receive about 40 mg of folic acid per 500- to 1000-pound **equine** .

...in an aqueous medium to provide a mixture that can be administered to the affected **equine** , usually by mouth.

Exemplary or Independent Claim(s):

1. A method of treating **equine** protozoal myeloencephalitis (**EPM**) comprising administering to an **equine** suspected of suffering from **EPM** therapeutically effective amounts of pyrimethamine and a sulfonamide, wherein the relative weight ratio of pyrimethamine...

Non-exemplary or Dependent Claim(s):

...The method of claim 2 which inhibits the growth of an organism from the genus **Sarcocystis** .

...is administered in a daily dosage of about 15 to about 30 mg/kg of **equine** .

...

...folic acid is administered daily at a dosage of about 40 mg per 1000-pound **equine**

10/3,KWIC/22 (Item 17 from file: 654)
DIALOG(R)File 654:US Pat.Full.

4631468
Derwent Accession: 2000-571969
Utility
CERTIFICATE OF CORRECTION
C/ Antigen **test to detect** equine **protozoal myeloencephalitis in horse serum and cerebrospinal fluid**
; AN IMMU NOASSA **Y DETECTION METHOD AND KIT TO DIAGNOSE HORSES INFECTED WITH** SARCOCYSTIS **NEURONA USING POLYCLONAL OR MONOCLONAL ANTIBODIES AGAINST THE PROTOZOAN** ANTIGEN ; **DIAGNOSTIC KITS**
Inventor: Mansfield, Linda S., Bath, MI
Rossano, Mary G., Mason, MI
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Vrable, Ruth A., Williamston, MI
Assignee: Board of Trustees of Michigan State University(02), East Lansing, MI
Michigan State University (Code: 55149)
Examiner: Park, Hankyel T. (Art Unit: 168)
Combined Principal Attorneys: McLeod, Ian C.

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Provisional				US 60-120831	19990219
				US 60-152193	19990902

Fulltext Word Count: 14935

Antigen **test to detect** equine **protozoal myeloencephalitis in horse serum and cerebrospinal fluid**

Abstract:
The present invention provides an immunoassay to detect identifying **antigens** in horses that are infected with **Sarcocystis** neurona. The immunoassay is preferably an **antigen** -capture-based assay that relies upon polyclonal or monoclonal antibodies against a 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** specific to **Sarcocystis** neurona to detect the presence of the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** in **equine** serum or **equine** cerebrospinal fluid.

Summary of the Invention:
...The present invention relates to an immunoassay to detect identifying **antigens** in horses that are infected with **Sarcocystis** neurona. The immunoassay is preferably an **antigen** -capture-based assay that relies upon polyclonal or monoclonal antibodies against the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** specific to **Sarcocystis** neurona to detect the presence of the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** in **equine** serum or **equine** cerebrospinal fluid. The present invention further relates to polyclonal and monoclonal antibodies against the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** , and DNA and clones encoding the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** . ...

... **Equine** protozoal myeloencephalitis (**EPM**) is a neurological disease caused by the protozoan parasite **Sarcocystis** neuronaa. In recent years, **EPM** has caused significant health, economic, and emotional costs to horses and their owners (reviewed by...

...Practicing Veterinarians 14: 1359-1366 (1997). Opossums have been implicated as the natural reservoir of **Sarcocystis** neurona because the sexual stages of the parasite occur in the intestines of the opossum...

...Horses accidentally eat the opossum feces containing the sporocysts when they are grazing; however, because **Sarcocystis** neurona does not appear to form mature tissue cysts in **equines** , **equines** are considered to be dead end hosts. Because opossums are ubiquitous in the United States... field or horse-side diagnostic tests for determining whether a horse is currently infected with **Sarcocystis** neuronaa. A Western blot test was developed to detect antibodies to **Sarcocystis** neurona in cerebrospinal fluid of horses suspected of having **EPM** ; however, these Western blot assays have not been reliable in predicting the presence of **Sarcocystis** neurona due to the prevalence in horses of cross-reacting antibodies to other **Sarcocystis** species (Granstom et al. J. Vet. Diag. Invest. 5: 88-90 (1993), Fenger et al...

...S. application Ser. No. 09/156,954 which reliably measures the prevalence of antibodies against **Sarcocystis** neurona in horse serum or cerebrospinal fluid. The improved method measures the presence of identifying 16 ([+-]4) and 30 ([+-]4) kDa **Sarcocystis neurona antigens** on Western blots that have been pretreated with antibodies against bovine **Sarcocystis** cruzi which prevents binding to the western blot of antibodies that may be present in the horse serum against **Sarcocystis** spp. other than **Sarcocystis neuronaa**

Description of the Invention:

...antibodies that recognize and react to the 16 ([+-]4) and/or 30 ([+-]4) kDa identifying **antigens** of **Sarcocystis** neurona...

... **Sarcocystis** neurona was cultured on **equine** dermal **cell** line cultures as taught in Example 5 or on bovine monocyte **cell** cultures as taught by Granstrom et al., J. Vet. Diagn. Invest. 5: 88-90 (1993). **Sarcocystis** neurona **merozoites** were harvested and the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** purified by methods known to the art for purifying **antigens** , i.e., the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** were purified from **merozoites** by two-dimensional polyacrylamide gel electrophoresis. Then the purified **antigens** are used to make monoclonal antibodies according to the methods in Antibodies, A Laboratory Manual...injection of 1.0 [mu]g of the 16 ([+-]4) or 30 ([+-]4) kDa identifying **antigen** per mouse mixed 1:1 with Titer max, Freund's incomplete adjuvant or Freund's complete adjuvant. After two weeks, a booster injection of 1.0 [DELTA]g of **antigen** is injected into each mouse intravenously without adjuvant. Three days after the booster injection a fusion is performed with a mouse myeloma **cell** line. Mid log phase myeloma **cells** are harvested on the day of fusion, checked for viability, and separated from the culture medium by low-speed centrifugation. Then the **cells** are resuspended in serum-free Dulbecco's Modified Eagle's medium (DMEM...

...20% fetal bovine serum, 1 mM pyruvate, 100 units penicillin, and 100 units streptomycin. The **cells** are released by perfusion with a 23 gauge

needle. Afterwards, the **cells** are pelleted by low-speed centrifugation and the **cell** pellet is resuspended in 5 ml 0.17 M ammonium chloride and placed on ice for several minutes. Then 5 ml of 20% bovine fetal serum is added and the **cells** pelleted by low-speed centrifugation. Afterwards, the **cells** are resuspended in 10 ml DMEM and mixed with myeloma **cells** to give a ratio of 3:1. The **cell** mixture is pelleted by low-speed centrifugation, the supernatant fraction removed, and the pellet allowed ...

...Finally, 10 ml of DMEM is added over a period of 2 minutes. Afterwards, the **cells** are pelleted by low-speed centrifugation and the pellet resuspended in DMEM containing 20% fetal...

...1 hypoxanthine, 0.5 [mu]M aminopterin, and 10% hybridoma cloning factor (HAT medium). The **cells** are then plated into 96-well plates...

...the plates is removed and replaced with fresh HAT medium. After 11 days, the hybridoma **cell** supernatant is screened by an ELISA ...previously described. In the ELISA assay, 96-well plates are coated with the appropriate identifying **antigen** . One hundred [mu]l of supernatant from each well is added to a corresponding well...

...removed to 2 cm[sup]2 culture dishes, with the addition of normal mouse spleen **cells** in HAT medium. After a further three days, the cultures are rescreened as above and those that are positive are cloned by limiting dilution. The **cells** in each 2 cm[sup]2 culture are counted and the **cell** concentration adjusted to 1X10[sup]5 **cells** per ml. The **cells** are diluted in complete medium and normal mouse spleen **cells** are added. The **cells** are plated in 96-well plates for each dilution. After 10 days, the **cells** are screened for growth. The growth positive wells are screened for antibody production those testing positive are expanded to 2 cm[sup]2 cultures and provided with normal mouse spleen **cells** . This cloning procedure is repeated until stable antibody producing hybridomas are obtained. Then the identified stable hybridomas are progressively expanded to larger culture dishes to provide stocks of the **cells** .

...

...prime the mice for ascites production. After 10 to 60 days, 4.5X10[sup]6 **cells** is injected intraperitoneally into each mouse and ascites fluid is harvested between 7 and 14 days later. Alternatively, the hybridoma **cells** are grown in culture according to methods well known in the art for cultivating hybridoma **cells** for antibody production...

...An alternate method for screening hybridomas for antibody production is as follows. **Sarcocystis** neurona is heat-denatured in 0.5 M Tris (pH 7.4) with 10% SDS, 20% glycerol and 5% 2-mercaptoethanol. The denatured **antigens** are separated by SDS-polyacrylamide gel electrophoresis in a 12-20% (v/v) linear gradient gel with a 4% (v/v) stacking gel. The separated **antigens** are electrophoretically transferred to PVDF membranes at 100 volts for 1.5 hours, then 150 stored frozen. Prior to use, the membranes are incubated with bovine serum albumen and **Sarcocystis** cruzi antibodies in Blocking buffer at a range of 1:10 to 1:100 ratio...

...produce monoclonal antibodies against various epitopes of the 16 ([+-]4) and 30 ([+-]4) kDa identifying **antigens** are expanded as above, and used to make monoclonal antibodies for the **antigen** -based immunoassay and for identifying cDNA library clones in Example 2 that contain **Sarcocystis**

neurona DNA which express either the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** .

...

...In the foregoing procedure, monoclonal antibodies against particular epitopes of the identifying **antigens** are produced...construction of a CDNA library that expresses the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** of **Sarcocystis** neuronaa. The methods for making and screening cDNA expression libraries are well known to those...

...the library for clones that express the 16 ([+-]4) and/or 30 ([+-]4) kDa identifying **antigens** . When polyclonal antibodies are used, the clones are preincubated with polyclonal antibodies against **Sarcocystis** cruzi to prevent binding of **Sarcocystis** neurona antibodies to any clone except for the clones expressing the identifying **antigens** .

...an ELISA-based assay kit for detecting the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** (**antigens**) in serum or cerebrospinal fluid from an **equine** . The assay demonstrates that a kit in the standard ELISA microtiter plate format is useful for detecting **antigens** in **equine** serum or cerebrospinal fluid that bind to the monoclonal antibodies made in Example 1 against the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigen** .

...

...are coated with monoclonal antibodies made against the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigen** as in Example 1, a series of wells are coated with bovine serum albumen for the negative control, and a series of wells are coated with **antigens** for the positive control. The **antigens** are affixed to the wells using methods well known in the art to immobilize **antigens** to microtiter wells. Such methods are disclosed in Antibodies, A Laboratory Manual, eds. Harlow and...

... **Equine** serum or cerebrospinal fluid, both neat and serially diluted from ...Next, alkaline phosphatase conjugated monoclonal antibody against the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** in Binding Buffer is added to the wells. The microtiter plate is incubated from 30...

...the serum or cerebrospinal fluid contains either the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** , a yellow color forms in the sample wells the color indicator. In which case, if a sample contains **Sarcocystis** neurona **antigens** , a blue-purple precipitate is formed. In an alternate embodiment further still, the antibodies are...

...5'-tetramethylbenzidine (TMB), odianisidine, and 5-aminosalicyclic acid (5AS), which provides in wells positive for **Sarcocystis** neurona **antigens** , green, orange, blue, yellow-orange, or brown, respectively...

...on a dip stick device for detecting the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** in serum or cerebrospinal fluid of **equines** is disclosed... the device. Serum or cerebrospinal fluid containing the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** form an antibody- **antigen** complex with the immobilized monoclonal antibodies whereas serum from an **equine** that does not contain the 16 ([+-]4) and/or 30 ([+-]4) kDa **antigens** does not form an antibody- **antigen** complex. The antibody- **antigen** complex is detected as described in U.S. Pat. No. 5,620,845 to Gould...This example provides a simplified method for the isolation, excystation, and culture of **Sarcocystis** species using opossums as a model. The method is an improvement over the isolation, excystation...

...Twenty-seven opossums from southern Michigan were humanely killed and their intestines screened for **Sarcocystis** spp. oocysts between Ap. 1996 and Mar. 1998. In addition, **Sarcocystis** oocysts collected from wild grackle (Quiscalus sp.) fed opossums and oocysts collected from wild-caught...

...the large intestine was removed from each positive animal and tested for the presence of **Sarcocystis** spp. sporocysts and other parasite ...The improvement in the excystation and culture of **Sarcocystis** sp. over the prior art is the mechanical excystation step as set forth below. The...by moving the slides back and forth. The material on the slides was washed with **cell** medium into flasks of confluent **equine** dermal **cells** (ATCC CCL-57, freely available from the American Type Culture Collection, 10801 University Boulevard, Manassas...

...units/ml), amikacin (100 [mu]g/ml), and amphotericin B (1.25 [mu]g/ml). **Sarcocystis** neurona isolated from neural tissue of **EPM** -affected horses could be passaged continuously long term on this **cell** line. Before and after inoculation, **equine** dermal **cells** were grown at 37[degree(s)] C. with 5% CO[sub]2, with medium changed...

...weekly thereafter. After inoculation, cultures were observed weekly for evidence of cellular damage due to **Sarcocystis** spp. replication and for the presence of extracellular **merozoites** using an Olympus CK2 inverted microscope. Positive cultures were confirmed by Romanowsky (modified Giemsa-Wright)-stained cytospin of infected **cells** using a Shandon Cytospin 3 centrifuge and a Wescor 7100 Aerospray slide stainer. Separate sterileIsolation, excystation, and culture of opossum **Sarcocystis** spp. by the improved method shown herein resulted in viable organisms for all 7 animals...

...from the same Michigan farm on which two horses had been diagnosed with histopathologically confirmed **EPM** . Each opossum harbored a million or more oocysts in the small intestinal mucosa; however, fewer...

...Dounce homogenizer and subsequent pepsin-NaCl-HCl digestion broke down tissues but did not disrupt **Sarcocystis** oocysts, many of which were still attached to tissue fragments. Further digestion with sodium hypochlorite...in HBSS plus penicillin, streptomycin, and amphotericin B remained contaminated with bacteria. Inoculation of dermal **cells** with this contaminated material resulted in **cell** death. Culture and sensitivity testing proved the contaminating organism to be Alcalcigens sp. Amikacin (100...

...substituted for the streptomycin in the mucosal preparation and in all subsequent solutions, including the **cell** growth media. Amikacin killed the contaminant and no bacterial contamination of any subsequent isolates using...

...Successful culture of **merozoites** from the first opossum isolate occurred in 13 of 15 flasks into which sporocysts pretreated...at only one site. Successful culture was further confirmed by Romanowsky-stained cytospin of infected **cells** . All flasks negative for **merozoites** visually and by Romanowsky-stained cytospin of **cells** were discarded eight weeks after inoculation because longer term culture did not result in more...

...six specific pathogen-free opossums fed wild-caught cowbird were

successfully excysted and grown in **equine** dermal **cell** culture in our laboratory using this technique as were sporocysts thought to be **Sarcocystis** falcatula from opossums fed wild-caught grackle (these were wild-caught opossums testing negative for **Sarcocystis** by fecal flotation for three weeks prior to infection). The cowbird isolates have grown well long term in **equine** dermal **cells** . Marsh et al., J Parasitology 83: 1189-1192 (1997) have shown that an **equine** -derived **Sarcocystis** neurona isolate grew highly efficiently long term in **equine** dermal **cells** . The grackle-fed opossum isolate (**Sarcocystis** falcatula) grew in **equine** dermal **cells** but only for a brief time, 3 to 8 weeks in three different infection trials. Although the **cell** line was not effective for long-term growth of this **Sarcocystis** sp., the excystation method and initial culture were successful...

...Thus, this example shows that multiple isolates of **merozoites** have been successfully cultured from opossum-derived **Sarcocystis** spp. oocysts using the improved method of digestion followed by manual excystation. Long-term growth of all opossum **Sarcocystis** spp. should be possible using the improvement and the appropriate **cell** line. **Equine** dermal **cells** work well for **Sarcocystis** neuronaa, but other **cell** lines may be more useful for other **Sarcocystis** spp. A more complete understanding of the life cycle of **Sarcocystis** neurona and, therefore; of the factors that determine exposure of horses should be possible using This example provides three chemical excystation methods for preparing **Sarcocystis** sp. oocysts. The chemically prepared samples were compared to samples prepared by the improved method...This examples shows the isolation of stage specific **Sarcocystis** neurona according to the following method which demonstrates the collection of intermediate stage **Sarcocystis** neurona for presence of **Sarcocystis** **sarcocysts** , which appear like grains of rice on the surface of the muscle. The **sarcocysts** were collected and extracted as follows...

...mM Tris HCl, 25 mM EDTA, 0.5% sodium dodecyl sulfate, and 2 mg of **proteinase** K (Boehringer-Mannheim). The samples were incubated overnight in a shaker at 50[degree(sThe nucleic acid solutions from the **sarcocysts** were tested for identity to **Sarcocystis** neurona using a **Sarcocystis** neurona specific PCR test using primers specific for **Sarcocystis** neurona SSURNA gene. The primers were the 3870R **Sarcocystis** neurona reverse primer, 5'-CCATTCCGGACGCGGGT-3' (SEQ ID NO:1), and the 1055 eukaryote universal...

...SEQ ID NO:2). These primers produced a 484 bp product when applied to a **Sarcocystis** neurona template. Another set of primers were used to verify the presence of SSURNA DNA...
...five 10-fold dilutions of DNA made from 1X10[sup]5 to 1X10[sup]1 **merozoites** in a sample to determine sensitivity. Annealing temperature was varied between 57 and 64[degree...

...concentration was tested at 1, 2, 3, and 4 mM. Samples which tested positive for **Sarcocystis** neurona and no other **Sarcocystis** sp. were fed to pathogen-free opossums. About one month later sporocysts were collected from the small intestine of the inoculated opossums and used to inoculate **equine** dermal tissue culture **cells** as descibed previously

Exemplary or Independent Claim(s):

1. In a method for detecting the presence of **Sarcocystis** neurona in an **equine** in an immunoassay, the improvement which comprises reacting

a biological sample from the **equine** suspected of harboring the **Sarcocystis** neurona with at least one isolated antibody specific for a 16 ([+-]4) kDa **Sarcocystis** neurona **antigen** and at least one isolated antibody specific for a 30 ([+-]4) kDa **Sarcocystis** neurona **antigen** , wherein each antibody binds its respective **antigen** to form an antibody- **antigen** complex.

Non-exemplary or Dependent Claim(s):

2. The method of claim 1, wherein the antibody- **antigen** complex is detected with a labeled antibody against the **antigen** or the antibody in the antibody- **antigen** complex...

...wherein the biological sample is selected from the group consisting of serum, cerebrospinal fluid, and **cell** culture fluid from **equine** dermal **cells** infected with **Sarcocystis** neurona from a biological sample6. The method of claim 1 wherein the antibody against the **antigen** is immobilized on a support selected from the group consisting of a membrane or a...

...7. In a method for detecting the presence of **Sarcocystis** neurona in an **equine** in an immunoassay, the improvement which comprises reacting a biological sample from the **equine** suspected of harboring the **Sarcocystis** neurona with at least one monoclonal or isolated polyclonal antibody specific for a 16 ([+-]4) kDa **Sarcocystis** neurona **antigen** and at least one monoclonal or isolated polyclonal antibody specific for a 30 ([+-]4) kDa **Sarcocystis** neurona **antigen** , wherein each monoclonal antibody binds its respective **antigen** to form an antibody- **antigen** complex...

...8. The method of claim 7 wherein the antibody- **antigen** complex is detected with a labeled antibody against the **antigen** or the antibody in the antibody- **antigen** complex...wherein the biological sample is selected from the group consisting of serum, cerebrospinal fluid, and **cell** culture fluid from **equine** dermal **cells** infected with a biological sample...

...12. The method of claim 7 wherein the monoclonal antibody against the **antigen** is immobilized on a support selected from the group consisting of a membrane or a...14. A method for detecting **Sarcocystis** neurona in an immunoassay comprising...

...a) reacting a biological sample from an **equine** suspected of harboring the **Sarcocystis** neurona with at least one monoclonal antibody specific for a 16 ([+-]4) kDa **Sarcocystis** neurona **antigen** and at least one monoclonal antibody specific for a 30 ([+-]4) kDa **Sarcocystis** neurona **antigen** wherein each monoclonal antibody is immobilized on a support and wherein each monoclonal antibody binds its respective **antigen** to form a complex, and...method of claim 14 wherein the complex is detected by a labeled antibody against the **antigen** or monoclonal antibody in the complex...wherein the biological sample is selected from the group consisting of serum, cerebrospinal fluid, and **cell** culture fluid from **equine** dermal **cells** infected with **Sarcocystis** neurona from a biological sample ...

...20. The method of claim 14 wherein the monoclonal antibody against the

antigen is immobilized on the support selected from the group consisting of a membrane or a...

...21. A kit for detecting **Sarcocystis** neurona in a biological sample from an **equine** comprising...

...a) at least one monoclonal or isolated polyclonal antibody against a 16 ([+-]4) kDa **Sarcocystis** neurona **antigen** and at least one monoclonal or isolated polyclonal antibody against a 30 ([+-]4) kDa **Sarcocystis** neurona **antigen** , wherein each antibody binds its respective **antigen** to form a complex...

...b) a positive control comprising the 16 ([+-]4) kDa **Sarcocystis** neurona **antigen** and a positive control comprising the 30 ([+-]4) kDa **Sarcocystis** neurona **antigen** ; and...

...c) a reagent for detecting the complex formed between the antibody and the **Sarcocystis** neurona **antigen** .

...

...21 wherein the reagent for detecting the complex consists of a labeled antibody against the **antigen** or antibody in the complex...24 wherein the biological sample is from the group consisting of serum, cerebrospinal fluid, and **cell** culture fluid from **equine** dermal **cells** infected with **Sarcocystis** neurona from a biological sample ...

...26. The kit of claim 21 wherein the monoclonal antibody against the **antigen** is immobilized on a support selected from the group consisting of a membrane or a...28. A kit for the detection of disease caused by **Sarcocystis** neurona in an **equine** which comprises...

...a support with a monoclonal antibody against a first epitope of a 16 ([+-]4) kDa **Sarcocystis** neurona **antigen** and a monoclonal antibody against a first epitope of a 30 ([+-]4) kDa **antigen** immobilized on a surface of the support to bind the **antigen** in a biological sample from the **equine** ; ...

...b) a first labeled monoclonal antibody against a second epitope of the 16 ([+-]4) kDa **antigen** and a second labeled monoclonal antibody against a second epitope of the 30 ([+-]4) kDa **antigen** to bind the **antigen** bound by the monoclonal antibody immobilized on the membrane; and...reagent for detection of the first labeled monoclonal antibody bound to the 16 ([+-]4) kDa **antigen** and a reagent for detection of the second labeled monoclonal antibody bound to the 30 ([+-]4) kDa **antigen** .

...

...wherein the biological sample is selected from the group consisting of serum, cerebrospinal fluid, and **cell** culture fluid from **equine** dermal **cells** infected with **Sarcocystis** neurona from a biological sample...

...32. The method of claim 28 wherein the monoclonal antibody against the **antigen** is immobilized on the support selected from the group consisting of a membrane or a...

...33. A monoclonal antibody against a 16 ([+-]4) kDa **antigen** of **Sarcocystis** neurona...

...34. A monoclonal antibody against a 30 ([+-]4) kDa **antigen** of **Sarcocystis** neurona...
...35. An isolated DNA encoding a 16 ([+-]4) kDa **antigen** of **Sarcocystis** neurona36. An isolated DNA encoding a 30 ([+-]4) kDa **antigen** of **Sarcocystisneurona** .

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Utility
REASSIGNED
C/ **Treatment of** equine **protozoal myeloencephalitis** ; VETERI **NARY MEDICINE; SYNERGISTIC MIXTURE OF PYRIMETHAMINE, SULFONAMIDE AND TRIMETHOPRIM**
Inventor: Russell, Meri Charm, Des Moines, IA
Fenger, Clara K., Lexington, KY
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Blue Ridge Pharmaceuticals Inc (Code: 55591)
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Law Firm: Howrey Simon Arnold & White, LLP

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Treatment of equine **protozoal myeloencephalitis**...

Abstract:
The present invention relates to compositions and methods for treating **equines** , such as horses, afflicted with **equine** protozoal myeloencephalitis or **EPM** . The therapeutic compositions comprise a combination of pyrimethamine and a sulfonamide, preferably, sulfadiazine, in the...

Summary of the Invention:
...The present invention relates to compositions and methods for treating **equines** , such as horses, afflicted with **equine** protozoal myeloencephalitis or **EPM** . **EPM** is a debilitating neurologic disease of **equines** which can affect the brain, the brain stem, spinal cord, or any combination of these three areas of the **equine** 's central nervous system. **EPM** is caused by infection by the protozoan parasite **Sarcocystis neurona** (recently referred to as **Sarcocystis** falcatula). There is no **vaccine** or approved animal drug product available for effectively treating this disease in horses...

...Although the symptoms and effects of **EPM** have been recognized since

the 1970's, it was not until 1991 that the protozoan parasite that causes **EPM** was cultured from a horse and given the name **Sarcocystis** neurona. The horse is an aberrant, dead-end host, as infectious forms of the parasite... **EPM** occurs in much of North America. Serologic surveys conducted in central Kentucky, one county in...

...environment was associated with a decrease in the numbers of horses exposed to the parasite. **EPM** appears to have a sporadic distribution, although outbreaks have occurred on farms in Kentucky, OhioA horse of any age, breed, or sex may be affected by **EPM** . The disease has occurred in a horse of two months of age, as well as...

...its thirties. In fact, any horse demonstrating neurologic abnormalities should be considered a candidate for **EPM** affliction...

...head tilt with asymmetry of the face (e.g., eyelid, ear, or lip). A severely **EPM** -affected horse may become recumbent and unable to rise. Lameness not traceable to orthopedic disease or any combination of the above signs may occur with **EPM** . Other unusual signs may also occur... Diagnosis of **EPM** is based on clinical signs and on testing of the horse's cerebrospinal fluid (CSF). Originally, the diagnosis was based on the presence of antibodies to **Sarcocystis** neurona in serum, though it is now known that a positive serum test cannot be...

...Currently available treatment of horses with **EPM** is expensive and typically requires a duration of at least ninety (90) days. In some... Adverse effects of therapy may include anemia, abortion, diarrhea and low white blood **cell** counts. Both medications for treatment of **EPM** inhibit folic acid metabolism. Unlike horses, however, the protozoan is unable to utilize pre-formed...16.7 mg/kg) and trimethoprim (3.3 mg/kg), to treat horses suffering from **EPM** . See, Welsch, B. B., in The Compendium North American Edition, **Equine** , Morris, D. D. (Ed.) (1991) pp. 1599-1602...of past and on-going effort, there remains an unfulfilled need for a treatment for **EPM** -afflicted **equines** , particularly horses, which is not only effective but is also convenient to administer to maximize...

...and reduce the emergence of resistant strains. In particular, prior compositions for the treatment of **EPM** involve three-component mixtures, including pyrimethamine, sulfadiazine and trimethoprim. Moreover, where prior compositions contained pyrimethamine...

...malaria only and hampering their usefulness in other pathological conditions, like protozoan-mediated diseases, especially **EPM** . The fact is that there is currently no approved drug or drug combination for the treatment of **EPM** ...Quite surprisingly, it has now been discovered that an effective, convenient method of treating **EPM** is realized by the administration to an **equine** suspected of being afflicted with **EPM** of therapeutic amounts of pyrimethamine and a sulfonamide, preferably sulfadiazine. The relative weight ratio of...

...the weight amount of sulfadiazine present. Preferably, the therapeutic compositions used for the treatment of **EPM** are substantially free of trimethoprim, most preferably having no trimethoprim at all. Similarly, the methods...

...not rely on the presence of significant amounts of trimethoprim in effecting successful treatment of **EPM** , using substantially the

pyrimethamine and a sulfonamide as the principal active ingredients against the pathologic agent, namely, the organism **Sarcocystis** neurona in **EPM** . Hence, the methods of the present invention do not include the co-administration of known...

...In a preferred embodiment of the invention, the afflicted **equine** , e.g., a horse, is given a daily dose of pyrimethamine, which is equivalent to about 0.8-1.2 mg per kg of **equine** , most preferably about 1.0 mg per kg. The subject is also given, concurrently for...

...day of a sulfonamide, which is equivalent to about 15-30 mg per kg of **equine** , most preferably about 20 mg per kg. Once daily administration of the active ingredients, say...

...It should be apparent that an object of the present invention is the treatment of **equine** protozoal myeloencephalitis or **EPM** by providing a veterinary composition comprising pyrimethamine and a sulfonamide, provided that the composition does...preferably, the veterinary composition of the present invention (or the instant method of treatment of **EPM**) is substantially free of trimethoprim...a sulfonamide, such as sulfadiazine, designed to overcome the shortcomings of currently available treatments of **EPM** and to provide a more effective drug combination for horses and other **equines** infected with an organism of the genus **Sarcocystis** . As previously mentioned, pyrimethamine may be given in a preferred dose of about 1 mg/kg **equine** with a sulfonamide in a dose of about 15 to 30 mg/kg. **equine** , preferably 20 mg/kg...

...described, below) daily on an empty stomach will provide adequate dosing for the treatment of **EPM** that neither pyrimethamine nor sulfadiazine can treat alone. Since **EPM** is a protozoal infection of the central nervous system, the appropriate drug combination must penetrate... composition may be prepared in unit dosage form depending upon the minimum size of the **equine** . Such unit dosage forms comprise a relative weight ratio of pyrimethamine to sulfonamide in a...

...The present invention has been found to successfully inhibit the growth of the organism **Sarcocystis** neurona in **equ**ines , such as mules, ponies and horses. It has been observed that the preferred sulfonamide, sulfadiazine...invention to employ compositions utilizing one or more sulfonamides and/or pyrimidine derivative in treating **EPM** . Examples of other suitable pyrimidine derivatives include, but are not limited to, 2,4-diamino...

Description of the Invention:

...Veterinary compositions effective for the general treatment of **EPM** are provided, below, in the form of an oral suspension. The amounts of each component...

...mentioned above, a useful dosage, e.g., for a 1,000 pound horse infected with **Sarcocystis** neurona (as evidenced by the presence of the protozoan in a sample from the subject...the subject may receive about 40 mg of folic acid per 500- to 1000-pound **equine** .

...in an aqueous medium to provide a mixture that can be administered to the affected **equine** , usually by mouth.

Exemplary or Independent Claim(s):

1. A method of treating **equine** protozoal myeloencephalitis (**EPM**)

comprising administering to an **equine** suspected of suffering from **EPM** therapeutically effective amounts of pyrimethamine and a sulfonamide, provided that if the sulfonamide is sulfadiazine...

Non-exemplary or Dependent Claim(s):
...8. The method of claim 1 which inhibits the growth of an organism from the **Sarcocystis** neurona...which the pyrimethamine is administered in a daily dosage of about 1 mg/kg of **equine** .
...

...is administered in a daily dosage of about 15 to about 30 mg/kg of **equine** .
...

...which the sulfonamide is administered in a daily dosage of about 20 mg/kg of **equine** ...folic acid is administered daily at a dosage of about 40 mg per 1000-pound **equine** .
...20. A veterinary composition in unit dosage form for the treatment of **equine** protozoal myeloencephalitis (**EPM**) comprising pyrimethamine and a sulfonamide in a relative weight ratio of about 1:10 to...

10/3,KWIC/30 (Item 1 from file: 349)
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01241209
VACCINE AND METHOD FOR TREATMENT OF NEURODEGENERATIVE DISEASES
VACCIN ET PROCEDE POUR TRAITER DES MALADIES NEURODEGENERATIVES
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Detailed Description

Detailed Description
... therewith.

5 ABBREVIATIONS: API-40, P-amyloid peptide 1-40; AD, Alzheimer's disease; APC, **antigen** -presenting **cell** ; CNS, central nervous system; Cop-1, Copolymer 1; DAT, dopamine transporter; HD, Huntington's disease; IRPB, interphotoreceptor retinoid-binding **protein** ; MPTP, 1-methyl phenyl-1,2,3,6-tetrahydropyridine; OHSC, organotypic hippocampal slice culture; PD, Parkinson's disease; PI, propidium iodide; RGC, retinal ganglion **cell** ; Treg, CD4+CD25+ regulatory T cells; TUNEL, terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling...

...or
aggregated proteins
For many decades, clinicians have been aware of the formation of insoluble **protein** aggregates in particular diseases. In Alzheimer disease (Selkoe, 1997, 2002), the presence in the CNS...

...with
I
neurodegeneration and dementia. Similarly, other neurodegenerative diseases have recently been discovered to involve **protein** aggregation in the brain. For example, prion diseases such as kuru, Creutzfeldt-Jacob disease and bovine spongiform encephalopathy are associated with amyloid deposits of the prion **protein** (PrP).

Polyglutamine repeat diseases such as Huntington disease are likewise associated with neuronal cytosolic and...

...in the cytoplasm of cells of the basal ganglia, include amyloid-like aggregates of the **protein** a-synuclein (Conway et al., 2000; Serpell et al., 2000).

Huntington's disease (HD), identified...

...identified as an unstable expansion of CAG repeats in the IT15 gene encoding huntingtin, a **protein** of unknown function (Menalled and Chesselet, 2002). The CAG repeat expansion results in an increased stretch of glutamines in the N-terminal portion of the **protein** , which is widely expressed in brain and peripheral tissues (Gutekunst et al., 1995). The exact...

...the CNS the local immune response, which is mediated by T cells directed against self- **antigens** residing in the site of the lesion (i.e., autoimmune T cells), determines the ability...

...by harnessing a peripheral adaptive immune response in the form of T cells specific to **antigens** residing in the site of damage (Hauben et al., 2000a; Moalem et al., 1999a; Yoles...

...protection are directed not against a particular threatening self-compound but rather against dominant self- **antigens** that reside at the lesion site (Mizrahi et al., 2002; Schwartz et al., 2003; Balcalash et al., 2002).

Further studies by the inventors suggested that T- **cell** specificity is needed in order to ensure that among the T cells that arrive at the site, those encountering their specific or cross-reactive **antigens** (presented to them by local microglia acting as APC) will become activated. The activated T...

...et al., 2003; Moalein et al., 2000; Kipnis et al., 2000).

The concept of T **cell** -dependent "protective autoimmunity" has been formulated by the inventor Prof. Michal Schwartz and her group...
...site. T cells homing to the lesion site are activated by cells presenting the relevant **antigen** . Once activated, they augment and control local immune cells, allowing efficient removal of toxic compounds ...

...the normal immune response. Based on this hypothesis, boosting the immune system with a suitable **antigen** should provide neuroprotection. Among suitable **antigens** identified by the present inventors is Copolymer 1.

Copolymer I
Copolymer 1, also called Cop...

...treatment of multiple sclerosis under the trademark Copaxoneg (Teva Pharmaceutical Industries Ltd., Petach Tikva, Israel).

Vaccination with Cop 1 or with Cop I-activated T cells have been shown by the...

...1, Cop I -related peptides and polypeptides and T cells activated therewith prevent or inhibit **neuronal** degeneration and promote nerve regeneration in the CNS or peripheral nervous system (PNS), and protect ...

...toxicity.

Prof. Schwartz and colleagues have shown that Cop 1 acts as a low-affinity **antigen** that activates a wide range of self-reacting T cells, resulting in neuroprotective autoimmunity that...

...degeneration (Kipnis et al., 2002a; Schwartz and Kipnis, 2002a). The neuroprotective effect of Cop I **vaccination** was demonstrated by the inventors in animal models of acute and chronic neurological disorders such...

...D) In another set of experiments mice were immunized in the flank with interphotoreceptor
binding **protein** (IRBP; 50 @tg) or S- **antigen** (50 gg) emulsified in CFA supplemented with 5 mg/ml of Mycobacterium tuberculosis. Control mice...

...Student's t-test; n = 6-8 mice in each group) or in the S-**antigenimmunized** group ($P < 0.0001$; n = 6-8 mice per group).

Figs. 11A-11F show that susceptibility of retinal ganglion cells to API-40 toxicity is T **cell** -dependent. (A) C57BL/6/J mice were injected intravitreally with 5 or 50 AM AP1...

...BALB/c/OLA mice
injected with API
Figs. 12A-12B show that immunization with an **antigen** residing in the site of toxicity rather than with the toxic agent itself protects against ...

...in C57131/6J mice. C57B1/6J mice were immunized in the flank with interphotoreceptor-binding **protein** (IRBP; 50 @tg) in CFA, the P-ainyloid peptide (1-40, non-aggregated) (50 gg...

...13A-13B show that passive transfer of activated splenocytes from mice immunized with dominant retinal **antigens** into naYve mice results in protection. (A) Wild-type C57131/6J mice were immunized in the hind foot pads with a combination of interphotoreceptor-binding **protein** (IRBP) and S- **antigen** (50 gg each) or 50 gg
OVA emulsified in CFA supplemented with 5 mg/ml of Mycobacterium tuberculosis. Ten days later draining lymph nodes were excised and pooled, **cell** suspensions were prepared, and the cells were counted. Cells were activated ex-vivo by stimulation with their specific **antigens** for 48 h, and the activated T cells were then injected i.p. into nalfve C57131/6J mice. T cells specific to IRBP + S- **antigen** were injected at a dose of 1.2 x 107 T cells in PBS. Within I h of passive T **cell** transfer the mice received an intravitreal injection of glutaniate (400 mnol), and 12
surviving retinal...

...OVA-specific T cells than in mice that received T cells specific to IRBP + S- **antigen** ($P < 0.001$; two-tailed Student's ttest). There was no difference between mice that...

...or to P-amyloid peptide (1-40, non-aggregated). One hour after this passive T- **cell** transfer, the mice were injected with a toxic dose of aggregated A01 Two weeks later...

...any peptide or polypeptide, including a random copolymer, that cross-reacts functionally with myelin basic **protein** (MBP) and is able to compete with MBP on the NMC class 11 in the **antigen** presentation.

The composition or vaccine of the invention may comprise as active agent a Cop...YFAK), and any other similar copolymer to be discovered that can be considered a universal **antigen** similar to Cop 1.
In another embodiment, the present invention relates to the treatment of ...

...principal excitatory neurotransmitter. However, in many neurodegenerative diseases, glutamate levels rise to toxic levels, causing **cell** damage. This model was therefore chosen to establish Cop 1 neuroprotective vaccination and optimize the...

...developed as a therapy for multiple sclerosis (MS), an autoimmune disease characterized by unregulated T-- **Cell** - -activity - against selfpeptides of the CNS. Cop I is given to MS patients once a...

...vaccination and the neuroprotective effect
Two ex vivo markers correlate with the efficacy - the T **cell** stimulation
index z@ild-inte-rfie-r'on--'y@-(IFN--y)-.- T-h-e-sti...

...immune response to Cop I vaccination was thus determined by in vitro evaluation of T- **cell** proliferation and the level-profile of cytokine secretion.

The effect of Cop 1 vaccination was...
...index (SI), where SI is the mean cpm of cells incubated in vitro with the **antigen** (Cop 1) divided by the mean **epm** of cells incubated in vitro without the **antigen** (Cop 1). A positive response was defined as SI>2. A single injection of Cop...

...days. These results are in agreement with the results obtained for neuroprotective efficacy and T- **cell** proliferation.

Neuroprotective efficacy was correlated with IFN-y secretion, similar to the effect shown in Fig 1. In contrast, T- **cell** proliferation remains high under daily injections of Cop This result shows that while Co -1...

...followed by the Fisher's least significant difference test.

Table 1. Effect of Cop I **vaccination** on survival of HD R6/2 mice
Cop 1 Cop I Cop 1
Control 75...

...0.065)
In conclusion, the results of Examples I and 2 show that Cop I **vaccination** attenuates **neuronal** **cell** death induced by exposure to elevated levels of the excitotoxic neurotransmitter glutamate, and that the...

...be said to have had some kind of impact on Huntington's disease.

SECTION II: **VACCINATION** WITH **AUTOANTIGEN** OR COP 1 PROTECTS AGAINST P-AMYLOID AND GLUTAMATE TOXICITY
Neurodegenerative diseases differ in etiology but are propagated similarly. In the experiments in this section, we show that **neuronal** loss caused by intraocular injection of aggregated P-amyloid was significantly greater in immunodeficient mice...

...augmented by elimination or addition, respectively, of naturally occurring CD4+CD25+ regulatory T cells (Treg). **Vaccination** with retina-derived **antigens** or with Copolymer-1, but not with P-amyloid, reduced the ocular **neuronal** loss. In mouse hippocampal slices, microglia encountering activated T cells overcame the cytotoxicity of aggregated Pamyloid. These findings support the concept of "protective autoinimunity", show that a given T **cell** -based **vaccination** is protective at a particular site irrespective of toxicity type, and suggest that locally activated...

...neurons - -Withstand -the ins It. Alil@jiiii
neurodegenerative diseases might be arrested or retarded by **vaccination** with Cop- I
or related compounds or by treatment with...occurring CD4+CD25+ regulatory T cells (Treg) or by evoking an immune response directed

against **antigens** derived from the tissue's own constitutively expressed proteins (rather than against the threatening compound...

...effect could be reproduced by passive transfer of T cells directed against the same self- **antigens** .

I 0
Materials and methods - Section 11
(vii) Animals. Mice were handled according to the...

...a lethal dose of pentobarbitone (170 mg/kg; C.T.S., Kiryat Malachi, Israel).

(viii) **Antigens** . Bovine interphotoreceptor retinoid-binding **protein** (IRBP) was purified from retinal extracts by affinity chromatography on Con A as described (Pepperberg et al., 1991). Bovine S- **antigen** (arrestin) was prepared from
the Con A column flowthrough by the method of Buczylko and...
...nmol; SigmaAldrich) or aggregated API-40 (50 @tM; Sigma-Aldrich).

(xii) Assessment of retinal ganglion **cell** survival. At the end of the experimental period the mice were given a lethal dose...

...eye was also counted, and served as an internal control.

(xiii) In-situ detection of **cell** death by terminal deoxynucleotidyl transferase DNA (TUNEL). Mice were killed 48 h after intraocular glutamate...

...34
then incubated for 10 min with PB S. For permeabilization, proteases were digested with **proteinase** K for 20 min at room temperature. The open ends of the DNA fragments were...

...spleens were harvested and mashed. T cells were purified (enriched by negative selection) on T **cell** columns (R&D Systems). The enriched T cells were incubated with anti-CD8 microbeads (Miltenyi...

...n-its-of mouse recombinant IL-2 (mrIL-2; R&D Systems).
(xvi) Preparation of **antigen** -specific activated lymphocytes from immunized mice. Ten days after immunization, the mice were killed and...

...fine wire mesh. The washed lymphocytes (2x 106 cells/ml) were activated with the relevant **antigens**
(IRBPI-20 or aggregated API-40, each at 10 @Lg/ml) in stimulation medium containing...

...Redding, Germany), beginning at the ventral surface. Slices containing the hippocampi were cultured on Falcon **cell** culture inserts, pore size 0.4 @im (Becton Dickinson), in 6-well plates. The
cultivation...

...with cultivation medium, and the slices were prepared for microscopy and visualized. To quantify neural **cell** death in the OHSCs, PI intensity in each slice was assessed by use of Image...

...two-tailed Student's t-test.

EXAMPLE 5. Retinal proteins can evoke a protective T **cell** -based response to glutamate intoxication.
We have shown previously that mice of different genetic backgrounds...

...attributed, at least in part,, to strainrelated variations in the ability to manifest a T **cell** -dependent protective response (Kipnis et al., 2001). In view of the observed failure of myelin...

...we were interested in examining whether immunization of mice with retinal proteins would improve their **neuronal** survival after exposure to glutarnate toxicity, and if so, whether the same **vaccination** would be effective against other threatening compounds (such as aggregated P-amyloid) injected into the...

...examined retinal cryosections subjected to terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL). Apoptotic **cell** death was observed in the
RGC
layer (Fig. I OA)-. -When-mice of this strain -were **vaccinated** with a hom o- -e nate
9
of whole retinal proteins (WIZ-H) in CFA...

...our hypothesis that the evoked protection against glutamate toxicity is an outcome of a T **cell** -mediated response to the retinal self- **antigens** , we examined whether immunization with the specific eyeI 0 resident **antigens** interphotoreceptor retinoid-binding **protein** (IRBP) or S- **antigen** (retinal arsenin), rather than with the retinal homogenate, can protect RGCs against glutamate toxicity. Immunization...

...0.0008; two-tailed Student's t-test; Fig.

10C). Immunization with the retinal self- **antigen** S- **antigen** in CFA resulted in a similar increase in neuronal survival relative to immunization with PBS...

...matched normal retinas.

It should be emphasized that the retinal self-proteins IRBP and S- **antigen** , both of which are capable of causing uveitis in susceptible mice (Caspi et al., 1990a...

...al., 2001).

38
EXAMPLE 6. Ability to withstand the toxicity of P-amyloid is T **celldependent** .

Having shown that the physiologically relevant **antigen** for protection against neurotoxicity is not the toxic compound itself (glutamate) but a self- **antigen** that resides in the site of damage, we then examined whether the same vaccination might...

...et al., 1998), and therefore its use allows us to further explore the concept of **antigenic** specificity. Surviving RGCs were counted I or 2 weeks after ocular injection of aggregated P...

...the ability of naYve mice to withstand the toxicity of aggregated API-40

is T **cell** -dependent, we compared RGC survival in wild-type-and nude (nu/nu) BALB/c/OLA...

...neurons survived in the injected wild-type mice (2316 1 53) than in their T **cell** -deficient counterparts (1779 1 147; P < 0.01). The 39
choice of BALB/c/OLA mice for this experiment was based on a previous observation that the T **cell** -dependent ability of this strain to withstand the consequences of CNS injury is significantly better...
...to the type of insult but to the ability to harness a well-controlled T **cell** -dependent immune response.

To further test our working hypothesis that the T **cell** specificity needed for neuroprotection is directed not against the threatening compound but against self1 5 **antigens** that reside in the site of the lesion, we subjected C57BL/6J mice to intraocular...

...1 45,
respectively; Fig. 12B).

To verify that the observed vaccination-induced protection is T **celldependent** , we prepared primary T cells directed against IRBP and S- **antigen** or against IRBP only. After their activation ex vivo, the lymphocytes (1.2x 107 cells...

...aggregated API Significantly more RGCs survived in mice that received lymphocytes activated with IRBP+S- **antigen** than in mice immunized with lymphocytes activated by the non-CNS **antigen** OVA (2220 1 38 compared tol652 1 563 P < 0.001; two-tailed Student's...

...active vaccination with IRBP (Fig. 12A) against the toxicity of aggregated API-40 was T **cell** -mediated.

The failure of T cells directed to the aggregated AD I-40 itself to...

...al., -unpublished observations). -To- fight -p-amyloid toxicity,- a@ more@ appropriate choice would therefore be **antigens** that reside in the site and can be presented to homing T cells.

4 1...

...that an appropriate choice for
vaccination in order to fight P-amyloid toxicity would be **antigens** that reside in the site of degeneration and that can be presented to the homing T cells. Because of the diversity of the human histocompatibility complex, vaccination with self- **antigens** cannot be assumed to be safe for therapeutic purposes. In searching for a safe vaccine we examined the efficacy of vaccination with the synthetic **antigen** glatiramer acetate(Cop-1) (Schori et al., 2001b; Kipnis et al., 2000) which was previously...

...intervention the ability of mice to withstand the toxicity of aggregated API-40 is T- **cell** dependent (Fig. 11) prompted us to investigate whether the ability of the neural tissue to...

...does not reflect the lack of M1IC-II expression, as this bioassay does not require **antigen** presentation.

EXAMPLE 9. In vivo animal test system for Alzheimer's disease
The beneficial effect...

...of one or more of the three major forms of the human P-amyloid precursor **protein** (APP), APP695, APP751, and APP770, or subfragments thereof, as well as various point mutations based...

...the eye, and protection against both of them was achieved by vaccination with the same **antigens** , namely peptides derived from proteins that reside in the eye. We interpret this finding as proof of principle that dominant self- **antigens** constitutively residing in a site of damage are the self-protective **antigens** against threatening conditions at this site. We further show that depletion of the naturally occurring CD4+CD25+ regulatory T cells (Treg) can increase the spontaneous response to such **antigens** and thus the ability to withstand the toxic effect of aggregated P-amyloid. As a therapeutic strategy, however, we propose vaccinating with Copolymer 1, a synthetic weak agonist of self- **antigens** (Schori et al., 2001b; Kipnis et al., 2000; Angelov et al., 2003; Ziemssen7et- al.- 2002)-, rdtlfdf -thal i With-the- - sfte---s-p-e-cific' s-elf- **protein** - s themselves, because the former can be used as a protective vaccine without risk of...

...of Alzheimer's disease, accumulation of aggregated API-40 is potentially a major cause of **neuronal** . toxicity (Hardy and Selkoe, 2002). The present results support the contention that the Pamyloid peptide...

...apparently induces microglia to adopt a cytotoxic phenotype. In addition, the failure of P-amyloid **vaccination** to protect against 0amyloid-induced stress in the eye is in line with observations from our laboratory that **cell** -surface NRIC-11 expression is impaired in microglia encountering aggregated P-amyloid (Butovsky et al...

...2003). Thus, T cells that can be locally activated, irrespective of the identity of the **antigen** (s) residing in the damaged site, can trasform the adjacent microglial population from an enemy...

...physiological weakening (but not blocking) of Treg might provide a way to boost the T **cell** -based self-defense.
It was shown by our group that the same autoimmune T cells...

...animals that are inherently susceptible to autoimmune disease the protocol used for eliciting the T **cell** response critically affects the outcome. Thus, a strong adjuvant might lead to an autoimmune response... 2004a). Moreover, in susceptible strains devoid of immune cells (SCID) and
thus- lacking a -T **cell** -based regulatory mechanism, passive transfer of encephalitogenic T cells causes EAE, but is not sufficient...

...susceptible strains without running the risk of negative side effects, the use of weak synthetic **antigens** such as Cop-1 or other related compounds deserves consideration. Such a strategy, unlike vaccination with a peptide derived from a toxic **antigen** such as Pamyloid, can potentially provide risk-free benefit. Moreover, the same safe **antigen** can be used for protection at different sites of degeneration, a situation that is often...

...the body harnesses the immune system for protection against neurodegenerative conditions is via a T **cell** -dependent pathway. In

addition, they strengthen the notion that in adopting a therapeutic approach to neurodegenerative diseases characterized by **protein** deposition, the **antigen** selected for vaccination should not be the diseasespecific **protein** such as the aggregated AP1-40 in Alzheimer's disease, Lewy bodies in Parkinson's disease, or prion **protein** (PrP) in prion disease (Dodart et al., 2003; White et al., 2003), but a peptide derived from an immunodominant self- **protein** that resides at the site of CNS damage, a cryptic self-peptide, or an altered...

...with self such as Copolymer 1 and Copolymer 1 -related peptides and polypeptides.

The T **cell** -based vaccination described in Section II above protected mice from- the neurdddg&hdrdtiV6 -effdcts'of...

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01106055
LIVE ANTENUATED PARASITE VACCINE
VACCIN DE PARASITE VIVANT ATTENUE
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Detailed Description
Claims

Detailed Description
... infects the host and unlike T. brucei, will multiply inside the host cytoplasm of different **cell** types. After rupture of the host **cell** new trypomastigote forms are released which can again be ingested by cone nosed bugs.

The...

...most frequently recorded disease affecting poultry kept in modern poultry industry.

5 The family of **Sarcocystidae** , comprising Toxoplasma, **Sarcocystis** and Neospora is also known to have pathogenic members.

Toxoplasma is a widespread parasitic infection...

...cause severe abortion storms in cattle, Another Neospora species, N. hughesi, is suspected to cause **equine** protozoal myeloencephalitis in horses.

Many **Sarcocystis** species are present in cattle, pigs, sheep, goats and horses.
Economically, Sarcopystis neurona is recognized as the most common cause of clinical **equine** protozoal myeloencephalitis in horses. In the U.S. 50% of horses are seropositive for S...

...they can induce humoral IgG and local IgA, they raise immune responses to many protective **antigens** , they provide a more durable immunity and are more cross-reactive. Moreover they are low...

...each and every parasite. For the production of killed vaccines, one needs to know which **antigens** must be left unaltered by the inactivation method for each and every parasite. And apart...

...of Trypanosomatidae, there is at least one moment in which a certain stage infects a **cell** of a host and starts dividing. It was now surprisingly found that if ribosome synthesis...

...stopped at or around the moment of infection, the parasite nevertheless does enter the host **cell** and divides several times using the present pool of ribosomes, thereby perfectly mimicking natural infection...

...will after some time unavoidably become extinct.
This goal was attained by placing a ribosomal **protein** gene under the control of an inducible promoter.

An inducible promoter is a promoter that...

...on and off.

Examples of such promoters will be given below.

In principle, each ribosomal **protein** gene can be used as a target, since in principle all ribosomal proteins are needed...

...Therefore, live attenuated parasites according to the invention can be obtained by placing a ribosomal **protein** gene under the control of an inducible promoter, regardless the fact if this ribosomal **protein** gene encodes a ribosomal **protein** to be incorporated in plastid-, 1 0 mitochondrial or cytoplasmatic ribosomes.

Ribosomal **protein** sequences are highly conserved between the various parasites.

Therefore, DNA probes of the ribosomal sequences...
...phylum 1 5 Apicomplexa and the family of Trypanosomatidae. Additionally, the sequences of many ribosomal **protein** genes for many different parasites can be found in the NCBI- **protein** database (http://www.ncbi.nlm.nih.gov).

The fact that the lack of one ribosomal **protein** can already disturb the formation of -stable ribosomes has been demonstrated in various plants, animals...

...Seaboe-Larssen & S., Lambertsson, A., Genetics 143: 877-885 (1996)). Another example is the ribosomal **protein** gene YS3 of yeast, which encodes the yeast ribosomal **protein** S3. Its disruption yields non-viable haploid spores of Saccharomyces cerevisiae (Finken-Eigen, M., Domdey...

...research communications 223, 397-403 (1996)). These studies demonstrated that down-regulating a single ribosomal **protein** can already affect the formation and/or proper functioning of ribosomal complexes.

The promoters to...

...used in parasites according to the invention for the control of transcription of the ribosomal **protein** gene need to fulfil only one prerequisite. They must be switched on during the propagation...

...of the gene it controls. In the present invention this gene would be a ribosomal **protein** gene. A promoter is switched off if transcription of the gene that it controls is...

...is no need for a complete inhibition of transcription anyway. A low level of ribosomal **protein** transcription will finally result in an 0 extended live span of the parasites, before they...

...recipient host that receives the parasite as a vaccine.

If necessary, two or more ribosomal **protein** genes can be placed under the control of inducible promoters. This would be a preferred...

...is a leaky promoter, or in the exceptional case that lack of one specific ribosomal **protein** is not sufficient to destabilize the ribosome.

The invention will be explained by the following...

...Humans and warm-blooded animals are the target mammals for vaccination, and therefore the Toxoplasma **tachyzoite** is the parasitic stage for which the live attenuated parasite is needed. Therefore, the **tachyzoite** is the parasitic stage in which, according to the invention, a ribosomal **protein** gene is brought under the control of an inducible promoter.

The thus made recombinant parasite...

...to the native situation. Therefore, the process of infection, and of invasion of the host **cell** will perfectly mimic the process of natural infection. As soon as the parasite starts dividing...

...of ribosomes over its progeny. Since the promoter of (at least) one of the ribosomal **protein** genes is however in the switched off position when in the host **cell** , there will be either reduced or even no de novo synthesis of ribosornes. Therefore, the...

...vaccines thus closely relates to that of Toxoplasma as described above. As for Toxoplasma, the **tachyzoite** is the parasitic stage in which, according to the invention, a ribosomal **protein** gene is brought under the control of an inducible promoter. The development of molecular genetics...

...13(2):123-33 (1997)) For the production of a life attenuated Eimeria parasite, the **merozoite** is the parasitic stage in which, according to the invention, a ribosomal **protein** gene is brought under the control of an inducible promoter. In this case, the vaccine does not comprise the **merozoite** however, but the sporulated oocysts. This is due to the fact that the sporulated oocyst...

...the parasite is normally ingested by the chicken. For the replication of the first recombinant **merozoites** made according to the invention, it suffices however to introduce these into the digestive tract...

...mosquito. The sporozoite infects the liver within two minutes after injection, to produce schizonts and **merozoites** . The **merozoites** , in turn, infect erythrocytes and replicate there. It is at this moment in time that...

...1995))
Live attenuated Theileria vaccines according to the invention can again be based upon recombinant **merozoites** . These **merozoites** can be grown and maintained in lymphocytes.

It is in the lymphocyte that the **merozoite** starts dividing, synchronously with the division of the lymphocyte, while a few free progeny parasites...

...11-20 (1996) and Hulliger, L. J. Protozool. 12: 649-655 (1965).

Live attenuated Babesia **vaccines** can be made using the **merozoites** and/or trophozoites for recombination. These can be cultured in erythrocytes. The whole approach is...

...above. See !.a. Levy, M.G and Ristic, M.

Science 207: 1218-1220 (1980).

For **Sarcocystis** species such as S. suihominis and S. **neurona** , both

the sporozoite and the **merozoite** are targets for recombination according to the invention. And again, the principle is the same: the recombinant sporozoite provides recombinant **merozoites** and these **merozoites** slowly become extinct due to lack of ribosomes in the absence of de novo ribosome protein synthesis. The recombinant **merozoites** can be used directly in a **vaccine** . See e.g. Murphy, A.J. and Mansfield, L.S. J. Parasitol. 85: 979-981...

...Biochem. Parasitol. 1 1 2: 61-69 (2001)), and can be adjusted to regulate ribosomal **protein** gene transcription as follows.

briefly, the procyclic form of the parasite is the target for...

...to the invention of the order of Kinetoplastida, first an extra copy of a ribosomal **protein** gene is inserted together with a promoter containing one or more tetracycline operator elements. Subsequently...

...is comparable to methods for Apicomplexa as described below. Direct targeting of the endogenous ribosomal **protein** genes is not feasible for Leishmania and Trypanosoma, because most genes in Leishmania and Trypanosomes...

...a vaccine according to the invention against e.g. Babesia can be based upon recombinant **merozoites** , this holds true for all Babesia species. Details concerning the life cycles of the various...

...or the family of Trypanosomatidae that have as a characteristic that they comprise a ribosomal **protein** gene under the control of an inducible promoter.
The concept of inducible promoters has already...

...2723-2729 (1995)) and the ecdysone-inducible expression system (Invitrogen) (Yao, T.P. et al., **Cell** 71: 63-72 (1992)).

In principle there are two kinds of inducible promoters: those that...

...repressor capable of reversibly binding said operator site. The binding and detachment of the repressor **protein** can then be regulated by the "condition" applied as mentioned above, i.e. the presence...

...integration of one or more tetracycline operator element(s) in the promoter of a ribosomal **protein** gene near the start of transcription.

The tet-repressor gene is a gene that encodes a **protein** capable of binding to the tetoperator site thus blocking transcription of the adjacent gene. This...

...the parasite using recombinant DNA techniques. Thus, the recombinant parasite will synthesize the tet-repressor **protein** . The tet-operator is preferably introduced in the vicinity of the transcription start site of one or more ribosomal **protein** genes, preferably in the endogenous promoter, upstream of the STS. The tet-repressor **protein** will consequently bind to the tet-operator, thus blocking the transcription of the downstream ribosomal **protein** gene. In the presence however of tetracycline, the repressor will detach from the tet-operator...

...chicken). The following should be noted: tetracycline is taken up by extracellular and intracellular parasites. **Cell** rupture of the host **cell** is not required for the drug to have 1 0 effects on the regulation

of...tetR-system is used as an inducible promoter system, the promoter upstream of the ribosomal **protein** gene can e.g. be the endogenous promoter, now made inducible by cloning the tet...

...that any other promoter capable of providing a sufficiently high transcription level of the ribosomal **protein** gene is also suitable.

If another inducible promoter system is used, it would be easy...

...that any other promoter capable of providing a sufficiently high transcription level of the ribosomal **protein** gene cloned downstream, is also suitable.

Step 2, the replacement of a wild-type ribosomal **protein** gene with one containing one or more tetO sites (= tet-operator sites) in the vicinity ...

...STS requires the insertion of the tet-operator site between the promoter of the ribosomal **protein** gene of choice and the gene itself. The tet-operator has been described by Yan...

...0 In principle, insertion of a single tet-operator site in front of the ribosomal **protein** gene of choice would suffice. The tetR-system, is, as all biological systems, however not...

...described how to locate such STS.

The step of replacement of a wild-type ribosomal **protein** gene with a recombinant gene comprising one or more tet-operator sites can La. be...

...form of this embodiment, the attenuated live parasite belongs to the family Eimeridiidae, Cryptosporidiidae or **Sarcocystidae** .

In an even more preferred form of this embodiment, the attenuated live parasite belongs to the genus Eimeria, Cryptosporldium, Toxoplasma, **Sarcocystis** or Neospora.
In another more preferred form of this embodiment, the attenuated live parasite belongs...

...species Trypanosoma brucei or T.

cruzi
In another preferred form of this embodiment, a ribosomal **protein** gene of the live attenuated parasite according to the invention is under the control of...

...anhydrotetracyclin, or derivatives 1 0 thereof.

In another preferred form of this embodiment, the ribosomal **protein** gene of choice is the gene encoding L9, S3, plastid-S9 or SI 3, preferably...

...SI 3 of Toxoplasma gondii.

The nucleotide sequence of the gene encoding Large subunit ribosomal **protein** number 9 (L9), as well as upstream sequences comprising the promoter region is depicted in...
...4831 stop TAA stopcodon

The nucleotide sequence of the gene encoding plastid Small subunit ribosomal **protein** number 9 (S9), as well as upstream sequences comprising the promoter region is depicted in...

...4338 3' utr 3'UTR
The nucleotide sequence of the gene encoding Small subunit ribosomal **protein** number 13 (S13), as well as upstream sequences comprising the promoter region is depicted in...

...3490 3639 e exon 3
The nucleotide sequence of the gene encoding Small subunit ribosornal **protein** number 3 (S3), as well as upstream sequences comprising the promoter region is depicted in...

...may also comprise a so-called "vehicle". A vehicle is a compound to which the **protein** adheres, without being covalently bound to it. Such vehicles are i.a. lipid vesicles, ISCOMs...

...very suitable as vaccine vehicles.
A special form of such a vehicle, in which the **antigen** is partially embedded in the vehicle,
is the so-called ISCOM (EP 109.942, EP...

...gene in such a way that it corresponds to the codon usage of the eukaryotic **cell** , thus arriving at a synthetic tet-repressor gene.

This has been done by Meissner M...

...gene could not be further optimised, since it is already fully adapted to the eukaryotic **cell** . Moreover, one would expect this "synthetic" tet-repressor **protein** to be the best suitable repressor **protein** in the eukaryotic **cell** . This **protein** is in principle the same **protein** as the native **protein** , and thus by definition best fitted for interaction with the tet-operator site.

It was...

...repressor provide a significantly better regulation of the tet-operator than even the tet-repressor **protein** encoded by a fully eukaryote-adapted "synthetic" tetrepressor gene.
Thus, such fusion proteins would be...

...This is even more an unexpected finding because 3D-structure studies of the tet-repressor **protein** would predict that Nterminal fusion would negatively interfere with DNA-binding. This was however surprisingly...

...to be the case in practice.

A heterologous gene is any gene that encodes a **protein** other than the tet-repressor **protein** . A heterologous **protein** is any **protein** other than the tet-repressor **protein** . A recombinant gene is any artificially made gene that comprises (part of) a heterologous I...

...that side of the tet-repressor gene that encodes the N-terminus of the tetrepressor **protein** .

The fusion **protein** must be able to reach the nucleus in order to

interact with the tetoperator. Therefore there are a number of prerequisites to be fulfilled by the tet-repressor 5 fusion **protein** : the final molecular weight of the monomeric tet-repressor fusion **protein** must be <60 kD, the heterologous part of the fusion **protein** must be on the N-terminal side of the tet-repressor **protein** , and the fusion **protein** must be free of GPI-anchors, secretion/excretion signals and trans-membrane regions. In principle, each and every **protein** or part thereof that meets with these prerequisites and (as a consequence) is capable of targeting the nucleus can be used for N-terminal fusion with the tet-repressor **protein** .

There is no need to use a full length heterologous **protein** for fusion. It suffices to use a part of such a heterologous **protein** . A part is considered to be a fragment of at least 1 0 amino acids, preferably a least 20 amino acids as the heterologous fusion **protein** .

Preferably, the part originates from the N-terminal side of the heterologous **protein** .

Heterologous proteins of choice are e.g. Green, Red and Yellow Fluorescent **protein** and the CAT- **protein** .

Therefore, another embodiment of the present invention relates to DNA-fragments encoding a tet-repressor fusion **protein** that has as a characteristic feature that it comprises the tet-repressor **protein** and a heterologous **protein** or a part thereof, that is fused at the N-terminal side of the tet-repressor **protein** wherein the monomeric form of the fusion **protein** has a size of <60 kD and the fusion **protein** is free of GPI-anchors, secretion/excretion signals and trans-membrane regions.

Still another embodiment of the present invention relates to a tet-repressor fusion **protein** as such, that has as a characteristic feature that it comprises the tet-repressor **protein** and a heterologous **protein** or a part thereof, that is fused at the N-terminal side of the tetrepressor **protein** wherein the monomeric form of the fusion **protein** has a size of <60 kD and the fusion **protein** is free of GPI-anchors, secretion/excretion signals and transmembrane regions.

The membranes to which...

...trans-membrane regions" refers, are those membranes that are located between the cytoplasm of the **cell** and the outside world.

These membranes specifically exclude the membranes between the nucleus and the cytoplasm. Preferably, the tet-repressor fusion **protein** according to the invention does have some signals that specifically direct the fusion **protein** to the nucleus. This is clear, because the tet-repressor fusion **protein** (as is required for the native tet-repressor gene) has to enter the nucleus in...

...to its universal character, the combination of the tetR-systern and the tet-repressor fusion **protein** can be used not only in live attenuated parasites according to the invention, but certainly...

...parasites comprise the tet-operator combined with (the genetic information encoding) the tet-repressor fusion **protein** described above.

This allows an even better blocking and induction of the transcription of a...

...or derivatives thereof, comprise the tet-operator and the genetic information encoding a tetrepressor fusion **protein** as described above.

As will be shown in the examples, the unexpected characteristics of the tet-repressor fusion **protein** as described above are even more significant if two or more tet-operator sites are...

...according to the invention comprise not only the tetR-system and a tet-repressor fusion **protein** as described above, but also two or more tet-operator sites, instead of one.
EXAMPLES...

...promoter separated from the fusion of chloramphenicol acetyl transferase (CAT) coding sequence with green fluorescent **protein** coding sequence by a BgII1 site.

To obtain the ptubYFP/TR construct the CAT coding sequence was exchanged for yellow fluorescent **protein** (YFP) and the GFP coding sequence was exchanged for tet-repressor coding sequence (tetR). The...

...presented in Figure 1.

Example 2
Determination of the start transcription site of the ribosomal **protein** gene S13 of
Toxoplasma gondii
In order to determine the start of transcription of the ribosomal **protein** gene SI 3, RNA was isolated from Toxoplasma gondii RHAHXGPRT **tachyzoaftes** grown in Vero cells.

Using the GeneRacer@ kit (Invitrogen) gene specific full-length cDNA was ...
...Then the start of transcription (STS) could be determined. This was done for the ribosomal **protein** gene SI 3 using the following primers: REVI 3A (#7, SEQ ID NO: 1 1...

...as described by Roos, D.S. et al.

("Methods in Microbial Pathogenesis" In Methods in **Cell** Biology (1994), D.G. Russell, editor).

Selection of the stable transfectants was done according to...

...strain, transiently transfected with the LacZ gene under the control of the SI 3 ribosomal **protein** gene promoter. There is no tet-operator-site present in this construct.

b) SI 3i...

...strain7 transiently transfected with the LacZ gene under the control of the SI 3 ribosomal **protein** gene promoter, which additionally carries a tet-operator-site inserted at site +3 relative to...

...strain, transiently transfected with the LacZ gene under the control of the SI 3 ribosomal **protein** gene promoter which additionally carries a

tet-operator-site has been substituted at site -23...

...gene according to the invention.

As follows surprisingly from Figure 5, a fusion tet-repressor **protein** according to the invention gives a significantly better blocking of the transcription of LacZ when compared to the blocking found with synthetic tet-repressor **protein** (Meissner) as described above.

Also, surprisingly, a much better induction of LacZ transcription is found...

...repressor gene (Meissner) mentioned above.

Example 5
Insertion of tet operator elements in the fibosomal **protein** S13 locus using homologous recombination with the hit-and-run mutagenesis procedure.

To integrate a tet operator site on the genome at a specific locus, in this, case the ribosomal. **protein** S13 locus (S13), homologous recombination is required. For homologous recombination a large sequence part (in...

...6-thioxanthine against HXGPRT which results in loss of the pseudodiploid and creation of a **tachyzoite** with or without a tet operator site integrated at the S13 locus (-1:1 ratio...

...cassette under the control of a DHFR promoter. RNA was isolated from Toxoplasma gondii RH **tachyzoites** . This RNA was used for making cDNA using SUPERSCRIPT"m 11 RnaseH- Reverse Transcriptase (Gibco...

...Laboratory Press; ISBN: 0879695773). The HXGPRT coding sequence was amplified from the T. gondii RH **tachyzoites** 'cDNA using primers HXGPRT/BGLII-FW (SEQ ID NO: 28) and HXGPRT/PSTI-RV (SEQ...

...43/-23 relative to STS) was PCR amplified from genomic DNA of T. gondii RH **tachyzoites** using primers SI 3NOTI-FW (SEQ ID NO.

21) and S 1 3SACI-RV (SEQ...pminiHXGPRT.

Circular pS13s-23/pminiHXGPRT plasmid was electroporated as described previously (Example 4) into RHAHXGPRT **tachyzoites** . After infection into Vero **cell** monolayers, mycophenolic acid I xanthine selection was started as described by Donald et aL (1...

...Figure 3A: tetO insertions/substitutions in rp-S13 promoter.

Sequence of part of the ribosornal **protein** S13-promoter, also indicating the site of the +3 insertion and the -23 substitution, relative...

...level of LacZ expression. The labels of the horizontal axis indicate that 1.25xl 06 **tachyzoYtes** were used (50 % of originally made amount).

Figure 5: Determination of the LacZ expression level...

Claim

... phylum Apicomplexa or the family of
Trypanosomatidae, characterised in that said parasite comprises a ribosomal **protein** gene under the control of an inducible promoter. 2) Attenuated live parasite according to claim...

...2, characterised in that said parasite belongs to the family of the Eimeridiidae, Cryptosporidiidae or **Sarcocystidae** . 4) @ Attenuated live parasite according to claim 3, characterised in that said parasite belongs to the genus Eimeria, Cryptosporidium, Toxoplasma, **Sarcocystis** or Neospora. 5) Attenuated live parasite according to claim 2, characterised in that said parasite...

...characterised in that said inducible promoter is based upon an operator site and a repressor **protein** capable of reversibly binding said operator site. IO) Attenuated live parasite according to claims 1...

...13) Attenuated live parasite according to claims 1-1 2, characterised in that said ribosomal **protein** gene is the gene encoding L9, S3, plastid-S9 or S13, preferably L9, S31 plastid...
...1-13 and a
pharmaceutically acceptable carrier. 18) DNA-fragment encoding a tet-repressor fusion **protein** comprising the tet-repressor **protein** and a heterologous **protein** or a part thereof, said heterologous **protein** or a part thereof being fused to the N-terminal side of the tet-repressor **protein** , the monomeric form of said fusion **protein** having a molecular weight of less than 60 kD and being free of GPI-anchors...

...parasite comprises the tet-operator site and a DNA fragment encoding a tet-repressor fusion **protein** according to claim 18.) Attenuated live parasite according to claim 19, characterised in that said...

10/3,KWIC/33 (Item 4 from file: 349)

01083800 **Image available**
NUCLEIC ACIDS ENCODING SARCOCYSTIC **NEURONA** ANTIGEN **AND USES THEREOF**
ACIDES NUCLEIQUES CODANT L' ANTIGENE **<I>SARCOSYSTIS NEURONA</I> ET LEURS UTILISATIONS**
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LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SK

SL TJ TM TN TR TT TZ UA UG UZ VC VN YU ZA ZM ZW
(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT SE SI SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
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NUCLEIC ACIDS ENCODING SARCOCYSTIC **NEURONA** ANTIGEN **AND USES THEREOF**
ACIDES NUCLEIQUES CODANT L' ANTIGENE **<I>SARCOSYSTIS NEURONA</I> ET LEURS UTILISATIONS**

Fulltext Availability:
Detailed Description
Claims

English Abstract
The present invention provides novel isolated nucleic acids encoding **antigenic** proteins derived from **Sarcocystis** neurona, or unique fragments thereof In particular, the invention provides novel isolated nucleic acids encoding membrane-associated polypeptides SnSAG2, SnSAG3, and SnSAG 4. Also provided are purified **antigenic** polypeptide fragments encoded by the novel nucleic acid sequences set forth herein that encode for **Sarcocystis** neurona. In particular, the invention provides purified **antigenic** polypeptide fragments encoded by the novel nucleic acid sequences set forth herein that encode for SnSAG2, SnSAG3, and SnSAG 4. Further, the invention provides a purified **antigenic** polypeptide fragment encoded by the nucleic acid sequences set forth herein or a selective portion...

...carrier. Also provided isolated nucleic acids capable of selectively hybridizing with the nucleic acid from **Sarcocystis** neurona. The invention also provides vectors comprising the nucleic acids of the invention encoding **Sarcocystis** neurona or a unique fragment thereof and provides the vector in a host capable of...

...Finally, the invention provides a purified polyclonal and/or a monoclonal antibody specifically reactive with **Sarcocystis** neurona and a method of detection of **Sarcocystis** neurona utilizing the antibodies of the invention.

French Abstract
L'invention concerne de nouveaux acides nucleiques isoles codant des proteines **antigenes** derivees de <I>Sarcosystis neurona, </I>ou des fragments uniques de **celles** -ci. En particulier, l'invention concerne de nouveaux acides nucleiques isoles codant des polypeptides associes aux membranes SnSAG2, SnSAG3, et SnSAG 4. L'invention concerne egalement des fragments polypeptidiques **antigenes** purifies codes par ces nouvelles sequences d'acides nucleiques qui codent <I>Sarcosystis neurona</I>. En particulier, l'invention concerne des fragments polypeptidiques **antigenes** purifies codes par ces nouvelles sequences d'acides nucleiques qui codent pour SnSAG2, SnSAG3, et SnSAG 4. En outre, l'invention concerne un fragment polypeptidique **antigene** purifie code par les sequences d'acides nucleiques ou une partie choisie de **celles** -ci, dans un porteur pharmaceutiquement acceptable. Elle concerne egalement des acide nucleiques isoles capables de...

Detailed Description

... OR

ANTIHYPERTENSIVE AGENTS

Field of the Invention

The present Invention relates to nucleic acids of **Sarcocystis neurona** . In particular, the present invention relates to nucleic acids of **Sarcocystis neurona** and to nucleic acid reagents and antibodies for use in methods of detection and prevention of **Sarcocystis neurona** infection. More particularly, the present invention relates to novel nucleic acid sequences of **Sarcocystis neurona** and to utilization thereof including primers, probes, antigen/antibody diagnostic Idts, vectors for production of peptides encoding the novel nucleic acids, and to antigenic proteins and **vaccines** against **Sarcocystis neurona** .

Background of the Invention

1

Sarcocystis neurona is an apicomplexan parasite that is the primary cause of **equine** protozoal myeloencephalitis (**EPM**). Due to several factors, definitive pre-mortem diagnosis of **EPM** remains exceedingly difficult. In particular, the seroprevalence of S. **neurona** in horses is significant, yet the true incidence of **EPM** is quite low, thus indicating that infection does not equate with disease. Additionally, the immunoblot remains the only commercial assay available for testing samples from suspect **EPM** horses; while development of this test was a significant advance, it is a decade-old, first-generation assay that needs to be supplanted.

EPM is a common and debilitating infectious disease that affects the central nervous system of horses...

...Rooney et al., 1970), but it was not until 1991 that the etiological agent of **EPM** was isolated and designated S., neurona (Dubey et al., 199 1). S. neurona is related...

...growth in the intestinal epithelium of

2

their definitive host. Similar to other species of **Sarcocystis** , S. neurona has an obligatory heteroxenous life cycle, with the opossum (Didelphis virginiana) serving as...

...by ingesting sporocysts in feces from the opossum, but unlike the normal intermediate hosts, mature **sarcocysts** have not been found in **equine** tissues (MacKay et al., 2000); consequently, the horse is currently considered an aberrant dead-end...

...The geographic range of S.

neurona appears to be limited to the Western Hemisphere, thus **EPM** primarily affects horses in the Americas.

1 5

Recent seroprevalence studies found that a significant...

...1997), suggesting that these
animals are commonly exposed to the parasite. However, the incidence
of **EPM** is estimated to be below 1% (MacKay et al., 2000), indicating
3
that there is...
...S. neurona sporocysts gave
inconsistent results, and these studies were unable to authentically
reproduce acute **EPM** (Cutler et al., 2001; Fenger et al., 1997).

Consequently, it is apparent that other factors...

...infection are responsible for the progression to disease. It is
well established that a robust **cell** -mediated immune response is
important for controlling infections by coccidian parasites (Alexander et
al., 1997...

...et al., 2001 a; Marsh et al., 1997), and it is
possible that susceptibility to **EPM** may be increased in horses with
inappropriate and/or suppressed immune responses during S. neurona...

...induce a transient
1 5 immunosuppression has been shown to provide some improvement to
the **equine** challenge
model for **EPM** (Saville et al., 2001).

Definitive antemortem diagnosis of **EPM** remains exceedingly
difficult, for a variety of reasons. Horses afflicted with **EPM** exhibit
4
signs that are similar to a number of different neurological disorders
(MacKay et...

...not
equate to disease, since only a small proportion of seropositive horses
will suffer from **EPM** ; as a consequence, the detection of anti-S.

neurona antibodies in serum provides little diagnostic...

...antibody production, thus suggesting CNS
infection, has improved the predictive value of antibody detection for
EPM diagnosis. However, interpretation of CSF antibody presence can
be confounded by contamination of the CSF...

...assays are hampered by several intrinsic problems, and they provide
only mediocre predictive value for **EPM** diagnosis. Western blot
analysis (a.k.a., immunoblot) of crude S. neurona lysate remains the
immunodiagnostic test that is used to detect antibodies in suspect **EPM**
horses (Granstrom et al., 1993). The continued use of the immunoblot
has been necessitated by perceived **antigenic** cross-reactivity between
different species of **Sarcocystis** , and the assay relies on the
recognition
5
of several **antigens** , primarily in the low molecular weight range, by
serum/CSF antibodies (Dubey et al., 2001b...

...to improve the immunoblot test
have included the use of antibodies against the related parasite

Sarcocystis cruzi to block cross-reactive epitopes, theoretically increasing the specificity of the immunoblot analysis for...

...value. While the immunoblot has been utilized for a number of years to help diagnose **EPM** , it is a first-generation test that needs to be
replaced with improved assays based...

...false negative results.

Research efforts directed toward understanding immunity against S. neurona infection and improving **EPM** diagnosis have been somewhat hampered by the lack of molecular information for S.

neurona. The identification of S. neurona-specific **antigens** and characterization of the genes encoding these **antigens** as provided by the
present invention hereby allow for the production of recombinant parasite **antigens** via expression in E. coli and the subsequent generation
1 5 of monoclonal and monospecific polyclonal antibodies against the individual S. neurona **antigens** . The recombinant proteins and specific antibodies provided by the invention serve as valuable reagents for conducting immunological studies on S. neurona infections and the progression to **EPM** . Additionally, these reagents allow for the development of new and more reliable diagnostic tests; for example, a
7
recombinant S. neurona **antigen** furnishes the key component for a simple and efficient enzyme-linked immunosorbent assay (ELISA) that can be used to monitor specific antibodies in **equine** serum or CSF. As provided by the teachings herein, the development of an ELISA that is based on a single recombinant S. neurona **antigen** rather than whole parasite lysate provides a second-generation assay that significantly improves current methodologies for identifying S. neurona-infected animals. Notably, the use of a single **antigen** ELISA will allow for a more in-depth and complete dissection of antibody responses to...
...been simply
exposed to the parasite versus horses that are actively infected and suffering from **EPM** .

A fluctuating equilibrium is maintained between the **cell**
1 5 mediated and the hunioral (antibody) responses of the vertebrate immune system, and this...

...Finkelinan et al., 1990; Snapper et al., 1997). It is generally believed that a Thl **cell** -mediated response is necessary for control of coccidian parasites (Alexander et al., 1997; Krahenbuhl and...

...nature of the immune response (i.e., Thl versus
Th2) in S. neurona-infected and **EPM** horses. The selection of an **antigen** for development of a diagnostic test can be somewhat
9
subjective since any particular pathogen is composed of numerous **antigenic** proteins. Logically, the target molecule in a diagnostic assay
must unfailingly elicit a detectable antibody response in the infected animal. A number of previous studies have demonstrated that surface **antigens** of the Coccidia are exceedingly immunogenic. In particular,

the primary surface **antigens** of Toxoplasina gondii (Handman and Remington, 1980; Sharma et al., 1983) and Neospora caninum (Howe et al., 1998) have been shown to be immunodominant. These surface **antigens** , designated SAGs and SAG-related sequences (SRSs), have been implicated in'host **cell** attachment and invasion by the parasite (Dzierszinski et al., 2000; Grimwood and Smith, 1992; Hemphill...

...1994; Mineo et al., 1993), most likely through interactions with sulfated proteoglycans on the host **cell** surface (He et al., 2002; Jacquet et al., 200 1). In addition to their probable role as adhesins, there is increasing evidence that some of these surface **antigens** are involved in modulation of the host immune response (Lek-utis et al., 2001). Significantly, the TgSAG1 surface **antigen** of T.

gondii has been shown to protect mice against acute toxoplasmosis (Bulow and Boothroyd, 1991), and the NcSAG1 (p29) major surface **antigen** of N. caninum has been used to develop an ELISA for detection 10 of Neospora...

...SAGs are at least candidate proteins for the development of both diagnostic assays and protective **vaccines** . Prior to the present invention, however, it had not been shown that the surface antigens of S. **neurona** (i.e., SnSAG2, SnSAG3, and SnSAG4) are effective target molecules for examining immune responses in infected horses and for developing improved assays for **EPM** diagnosis. The present invention utilizes recombinant S. **neurona** SAGs that are provided by the invention to provide simple and reliable ELISAs, and these assays can be used to scrutinize specific hurnoral immune responses in **EPM** horses and for detecting the presence of S.

neurona in a test sample. Importantly, the developed ELISAs are valuable as tools to aid in the diagnosis of **EPM** infection in horses.

Nucleic acids of certain **Sarcocystis** and Toxoplasma species are known in the art. For example, Eschenbacher K-H et al. "Cloning and expression in Escherichia coli of cDNAs encoding a 3 1 -kilodalton surface **antigen** of **Sarcocystis** muris". Molec. Biochem. Parasitol. 1992, 53:159-168 (1992). Eschenbacher discloses the cloning and expression of a surface coat **protein** of **Sarcocystis** muris **merozoites** consisting of 11 amino acids with a predicted size of 31 kDa.

Velge-Roussel F...

...and Immunity, 2000, 68: 969-972, discloses that intra-nasal immunization with a SAG 1 **protein** derived from Toxoplasma gondii plus a cholera toxin provides protective immunity in mice. Specific cellular...

...this case the mouse, and can be partially controlled by i.n. immunization with the **protein** SAG 1 plus CT.

Nielsen et al. discloses the construction of a DNA vaccine using

the recombinant form of the surface coat **protein** SAG I in Toxoplasina gondii, consisting of 824-nucleotides encoding the 275 amino acid **protein** . Animals immunized with this plasmid produce anti-SAG1 antibodies which recognize the native SAG1. See...

...gondii SAG 1 used in vaccination had a significant protective effect against maternofetal transmission of **tachyzoites** . Absence of parasites in fetuses was denionstrated in 66-86% of fetuses from adult guinea...

...Bollen A, Beaumans R, Jacquet A. "Protective immunity against congenital toxoplasmosis with recombinant SAG 1 **protein** in a guinea pig model". Infect Immun. 2000 Sep;68(9):4948
Angus et al. discloses that immunization with a DNA plasmid encoding the SAG 1 (p30) **protein** of Toxoplasma gondii is immunogenic and protective in mice. Sera of immunized mice showed 14
recognition of T gondii **tachyzoites** by immunofluoreseence and exhibited high titers of antibody to SAG1 by ELISA. This data suggest that nucleic acid **vaccination** can provide protection against T. gondii infection in mice. See, Angus CW, Klivington-Evans D...

...rodents". J Infect Dis. 2000 Jan; 1 81(l):3 17
Fort Dodge Animal Health, " **Vaccine** Development" discloses that an S. **neurona** merozoite culture that is chemically inactivated and incorporates an adjuvant is used as an **EPM** **vaccine** . This **vaccine** has
been conditionally licensed for use but without any indication of its effectiveness in preventing Sarcocyst **neurona** induced **EPM** Fort Dodge Animal Health, " **Vaccine** Development" Discloses that an S.

neurona merozoite culture that is chemically inactivated and incorporates an adjuvant is used as the **EPM** **vaccine** . Fort Dodge Animal Health, 20001.

Other references of interest include:Buxton D. "Protozoan infections in...

...0,Donoghue PJ et al. "Attempted immunization of swine against acute sarcocystosis using cystozooite-derived **vaccines** ". Vet.

Immunol Immunopathol. 1985 Jan;8(1-2):83-92; Bulow R and Boothroyd J...

...TJ,
Marsh A, Greiner EC " S. falcatula from passerine and psittacine birds.

synonymy with S. **neurona** , agent of **EPM** ". J. Parasitol. 1995, Dec; 81(6):930-5; Mishima M, Xuan X, Shioda A, Omata...

...H, Mikami T. "Modified protection against Toxoplasina gondii lethal infection and brain cyst formation by **vaccination** with SAG2 and SRS 1 ". J Vet Med Sci. 2001 Apr; 63 (4):43 3...

...M, Hata H, Kobayashi M, Kiuchi M, Stauss HJ,
Yano A. "Protective immunity induced by **vaccination** with SAGI gene-transfected cells against Toxoplasma gondii infection in mice".

Microbiol Immunol. 1999;43(1):87-91; Artois M, Cliquet F, Barrat J, Schumacher CL."Effectiveness of SAG1 oral **vaccine** for the long-term protection of red foxes (Vulpes vulpes) against rabies". Vet Rec. 1997...

...Follmann EH, Ritter DG, Baer GM. "Evaluation of the safety of two attenuated oral rabies **vaccines** , SAW and SAG2, in 16 six Arctic mammals". **Vaccine** . 1996 Mar; 14(4):270-3; and Windeck T, Gross U." Toxoplasma gondii strain-specific...

...the foregoing art, there remains a need in the art for a safe and effective **vaccine** against **Sarcocystis** **neurona** . Likewise, as set forth above there is also a need in the art for diagnostic kits including antigen and antibody kits for fast and reliable diagnosis of **Sarcocystis** **neurona** infection.

Objects of the Invention
It is an object of the present invention to satisfy...

...providing a novel isolated nucleic acid capable of encoding 1 5 antigenic proteins derived from **Sarcocystis** **neurona** , or unique **antigenic** fragments thereof. It is also an object of the present invention to provide purified **antigenic** polypeptide fragments encoded by the novel nucleic acid sequences set forth herein that encode for **Sarcocystis** iieurona. In particular, it is an object of the present invention to provide a purified **antigenic** polypeptide fragment encoded by the 17 nucleic acid sequences set forth herein or a selective...

...invention to provide isolated nucleic acids capable of selectively hybridizing with the nucleic acid from **Sarcocystis** neurona including, but not limited to, primers and probes for utilization in polymerase chain reaction...
...Another object of the invention is to provide a vector comprising the nucleic acid encoding **Sarcocystis** neurona or a unique fragment thereof and to provide the vector in a host capable...

...object of the invention is to provide a purified antibody that is selectively reactive with **Sarcocystis** neurona or an immunodominant polypeptide provided by the invention or a genetic variant thereof. A...

...object of the present invention is to provide a purified monoclonal antibody specifically reactive with **Sarcocystis** neurona and a method of detection of **Sarcocystis** neurona 18 utilizing the antibodies of the present invention.

Summary of the Invention
The present...

...satisfies the need in the art by providing a novel isolated nucleic acid encoding an **antigenic** **protein** derived from **Sarcocystis** neurona, or a unique fragment thereof. In one embodiment,

the invention provides novel isolated nucleic acids encoding membraneassociated polypeptides SnSAG2, SnSAG3, and SnSAG 4.

The present invention also provides purified **antigenic** polypeptide fragments encoded by the novel nucleic acid sequences set forth herein that encode for **Sarcocystis** neurona. In one embodiment, the invention provides purified **antigenic** proteins or purified **antigenic**
1 5 polypeptide fragments encoded by the novel nucleic acid sequences set forth herein that...

...for SnSAG2, SnSAG3, and SnSAG 4. In another embodiment, the present invention provides a purified **antigenic** polypeptide fi-agment encoded by the nucleic acid sequences set forth herein or a selective...

...invention also provides isolated nucleic acids capable of selectively hybridizing with the nucleic acid from **Sarcocystis** neurona including, but not limited to, primers and probes for utilization in polymerase chain reaction...

...Further, the present invention provides vectors comprising the isolated nucleic acids set forth herein encoding **Sarcocystis** neurona or a
unique fragment thereof and provides the vector in a host capable of...

...present invention also provides a purified polyclonal and or a monoclonal antibody specifically reactive with **Sarcocystis** neurona and a method of detection of **Sarcocystis** neurona utilizing the
1 5 antibodies of the present invention,
Brief Description of the Drawings...

...is a sequence comparison of SnSAG1, SnSAG3, and SnSAG4 with TgSAG2E. The S. neurona surface **antigens** SnSAGI,
20
SnSAG3 and SnSAG4 are most similar to the TgSAG2 family of T.

gondi surface **antigens** . Sequence alignments of the predicted mature proteins revealed very moderate sequence identity (<25%). However, the...

...2 is a sequence comparison of SnSAG2 with TgSAG1 and TgSRS2. The S. neurona surface **antigen** SnSAG2 is most similar to the TgSAG1 family of T. gondii surface **antigens** . Similar to the other SnSAGs, SnSAG2 shares modest sequence identity to its TgSAG orthologues, but...

...TgSAGs.

Figure 3 shows a Western blot analysis of the Sn SAGs in S.

neurona **merozoites** . The SnSAG genes were expressed in E. coli, and monospecific polyclonal antisera were generated against the
21
recombinant proteins. Western blot analysis of reduced **antigen** revealed that each SnSAG migrated significantly higher than its predicted molecular weight, consistent with what...

...band at approximately
18-20 IdDa.

Figure 4 shows the SnSAGs are membrane-associated in **Sarcocystis** neurona merozites. Triton X-1 14 partitioning assays indicated that the SnSAGs are associated with...

...revealed that all four SnSAGs were separated exclusively into the detergent phase (D). The control **protein** , SnMIC IO, was partitioned into the aqueous phase (A), as expected.

Figure 5 shows that the four SnSAGs are displayed on the surface of **Sarcocystis** neurona **merozoites** . Surface biotinylation of S.

neurona **merozoites** indicated that the four SnSAGs are displayed on the
22
surface of the parasite. Western blot analysis with the SnSAG-specific antisera revealed each of the SnSAGs in the biotinylated **protein** fraction precipitated with immobilized streptavidin. The SnSAGs were not present in the non-labeled parasites, thus indicating that the streptavidin precipitation were specific for biotin-labeled proteins. The negative control **protein** (actin) was not detected in the biotinlabeled/streptavidin-precipitated **protein** fraction.

Detailed Description of the Invention
I 0
The present invention may be understood more...

...long felt need in the art by providing novel isolated nucleic acid sequences which encode **antigenic** proteins derived from **Sarcocystis** neurona, or which encode unique **antigenic** **protein** fragments thereof. As used herein, a "nucleic
acid" means a chain of at least two...

...nucleic acid is one that is substantially separated from other nucleic acid sequences in a **cell** or organism in which the nucleic acid naturally occurs. Likewise, by "isolated" nucleic acid is...

...and are meant to include genomic and subgenomic nucleic acids found in the naturally-occurring **Sarcocystis** neurona organism.

The nucleic acids contemplated by the present invention include a nucleic acid having sequences from which a **Sarcocystis** neurona cDNA can be transcribed; or allelic variants and/or homologs of thereof By "capable...

...hybridize with other nucleic acids to prevent an adequate positive hybridization with nucleic acids from **Sarcocystis** neurona and is meant to include stringent hybridization conditions including low, moderate and high stringency...
...the novel
sequences set forth herein or that can selectively hybridize with nucleic acids from **Sarcocystis** neurona. Modifications to the nucleic acids of the invention are also contemplated as long as...

...In particular, one embodiment of the present invention provides

isolated nucleic acid derived from three **Sarcocystis** neurona cluster sequences, namely Sn Cluster 144, Sn Cluster 21 and Sn Cluster 4, which...

...respectively and the sequences complimentary thereto . Also provided by the invention are the 26 corresponding **protein** or polypeptide amino acid sequences for these three **Sarcocystis** neurona cluster sequences. The polypeptide sequence comprising Sn Cluster 144 is set forth in the...

...the Sequence Listing as SEQ ID NO: 30. As used herein, the terms "polypeptide" and " **protein** " are used interchangeably and are meant to include any peptide-linked chain of amino acids...
...a polypeptide that has been substantially separated or isolated away from other polypeptides in a **cell** , organism, or mixture in which the polypeptide occurs.

Sarcocystis neurona is an apicomplexan parasite that can cause a severe neurologic disease in horses called **equine** protozoal myeloencephalitis (**EPM**). Similar to other members of the Apicomplexa, S. neurona is an obligate intracellular pathogen that...

...novel and undoubtedly important since they are responsible for the initial interactions with the host **cell** surface and host immune response. In Toxoplasma gondii for example, an extensive family of 25+ surface **antigens** has been identified, which are developmentally regulated and exhibit various levels of sequence similarity to either of the major T gondii surface **antigens** TgSAG 1 ...TgSAG2. These surface molecules appear to be involved in receptor/ligand interactions with the host **cell** surface, and there is increasing evidence that some of the T. gondii SAGs are involved...

...provides four isolated nucleic acids of 1 5 S. neuroiza (genes) that encode parasitic surface **antigens** . A sequencing project was conducted that generated approximately 8500 expressed sequence tags (ESTs) from this...

...of this sequence database has revealed a family of at least four S. neurona surface **antigens** that are orthologues of the SAG/SRS family of surface proteins in T. gondii. Based on their homology to the T gondii SAGs, 28 the novel S. neurona surface **antigens** have been designated SnSAG1, SnSAG2, SnSAG3, and SnSAG4 respectively. Each **protein** is predicted to contain an amino-terminal signal peptide and a carboxyl-terminal glycolipid anchor...

...assays confirmed that all four proteins are membrane-associated and displayed on the S. neurona **merozoite** surface (See, Figures 4 and 5) .

Additionally, these novel S. neurona proteins possess multiple conserved...

...1 and 2). Due to their surface localization

and relative homology to T gondii surface **antigens** , these S. neurona proteins have been designated SnSAG1, SnSAG2, SnSAG3, and SnSAG4.

Accordingly, one embodiment...

...ID NO: 21 comprises an 828 -nucleotide open reading frame of the SnSAGI gene of **Sarcocystis** neurona which encodes a 276 amino acid polypeptide set forth in the 29 Sequence Listing...

...addition site at the carboxy-terminal end (indicating surface localization).

Database searches with the predicted **protein** sequence of SnSAG1 (rSnSAGl) revealed significant similarity (alignment score = 80, E value = 2 x 10 -14) to a 31 kDa surface **antigen** from Sarcoeystis muris.

A recombinant form of the Sareocystis neurona SnSAG1 (rSnSAGl) has been expressed in E. coli. Western blot analysis of rSnSAG1 demonstrated that the recombinant **antigen** is recognized by antiserum from a rabbit that was immunized with S. neuro7za **merozoites** and by antibodies in cerebrospinal fluid (CSF) from an **EPM** (**Sarcocystis** neurona infected) horse (See, e.g., Figure 3).

1 5
Another embodiment of the present...

...ID NO: 23 comprises an 975 nucleotide open reading frame of the SnSAG2 gene of **Sarcocystis** neurona which encodes a 168 amino acid polypeptide set forth in the 30 Sequence Listing...

...ID NO: 25 comprises an 1585 nucleotide open reading frame of the SnSAG2 gene of **Sarcocystis** neuroiza which encodes a 281 amino acid polypeptide set forth in the Sequence Listing as...

...comprises an 1 1 1 1 nucleotide open reading frame of the SnSAG2 gene of **Sarcocystis** **neurona** which encodes a 287 amino acid polypeptide set forth in the Sequence Listing as SEQ...

...can be implemented into antibody-capture ELISAs and used to detect the presence of S. **neurona** antibodies in a sample. Likewise, the 31 recombinant proteins provided by the invention can be used as reagents for use in **vaccines** against S. **neurona** .

Another embodiment of the present invention includes the discovery of additional novel expressed sequence tags (EST) that encode novel antigenic peptides for utilization in the **vaccines** and diagnostic kits as disclosed by this invention.

In particular, in a presently preferred embodiment of the invention, cluster analysis of the **Sarcocystis** **neurona** expressed sequence tags (ESTs) generated from the cSn. I cDNA library has

revealed a gene family that encodes at least eight homologous proteins.

Of the approximately 8500 S. **neurona** ESTs that have been generated thus far, roughly 540 sequences can be placed in this gene family, which has been provisionally designated SnGF1 (S. **neurona** Gene Family 1). Based on its relative abundance in the collection of S.

neurona ESTs, SnGF1 encodes a set of similar proteins (at least eight) that are highly expressed and most likely play significant roles in the biology of S. **neurona** (i.e., parasite virulence factors). In addition to
their biological importance, the abundance of these...

...therefrom, make this gene family well
suited for the development of improved diagnostics and/or **vaccines** for **EPM** as set forth herein.

The eight SnGF1 isoforms identified thus far have been designated SnGFla...

...in the current public gene
databases, suggesting that SnGF I is relatively unique to S. **neurona** .

1 5
Accordingly, one embodiment of the present invention provides an isolated nucleic acid designated...

...acid reagents derived therefrom
which can be utilized to diagnose and prevent infection of S. **neurona** . Purified polypeptides encoded by the nucleic acids are also provided.

These polypeptides can be utilized in methods of diagnosis or as **vaccine** components for prevention of infection. Vectors are also provided which comprise the nucleic acids of...

...and prophylactic applications. The
36
present invention also provides purified antibodies selectively reactive with S. **neurona** . These antibodies can be used in various diagnostic methods or as a therapeutic.

In one embodiment, the invention provides purified **antigenic** polypeptides encoded by the nucleic acids set forth in the Sequence Listing. The invention also provides these **antigenic** polypeptides in a pharmaceutically acceptable carrier. The amino acid sequence of these polypeptides can be deduced from the nucleotide sequences set forth in the Sequence Listing.

Purified **antigenic** polypeptide fragments encoded by the nucleic acids of the present invention are also contemplated. As used herein, "purified" means the **antigen** is at least sufficiently free of contaminants
1 5 or **cell** components with which the **antigen** normally occurs to distinguish the **antigen** from the contaminants or components. Purified **antigenic** polypeptides of S. neurona and **antigenic** fragments thereof of
the present invention are also referred to herein as "the **antigen** " or "the

S. neurona **antigen** ." It is contemplated that the **antigenic** fragments can
be encoded from any portion of the nucleic acid encoding S. neurona as...

...24, 26
and 28 as described herein. Specifically, one example provides an approximately 12 kDa **antigenic** polypeptide encoded by an open reading frame of SEQ ID NO: 24 consisting essentially of...

...nucleotide as sequence set forth in the Sequence Listing as SEQ ID NO: 23.

An **antigenic** fragment of the **antigen** can be isolated from the whole **antigen** by chemical or mechanical disruption. The purified fragments thus obtained can be tested to determine their **antigenicity** and specificity by the methods taught herein. **Antigenic** fragments of the **antigen** can also be synthesized directly. An immunoreactive fragment is generally an amino acid sequence of at least about five 1 5 consecutive amino acids derived from the **antigen** amino acid sequence.

The polypeptide fragments of the present invention can also be recombinant proteins...

...by cloning nucleic acids encoding the polypeptide in an expression system capable of producing the **antigenic** polypeptide or fragments thereof.

38
Once the amino acid sequence of the **antigen** is provided, it is also possible to synthesize, using standard peptide synthesis techniques, peptide fragments chosen to be homologous to immunoreactive regions of the **antigen** and to modify these fragments by inclusion, deletion or modification of particular amino acids residues...

...sequences. Thus, synthesis or purification of an extremely large number of peptides derived from the **antigen** is possible.
The amino acid sequences of the present polypeptides can
I 0 contain an immunoreactive portion of the S. neurona **antigen** attached to
sequences designed to provide for some additional property, such as solubility. The amino acid sequences of an S. neurona **antigen** can include sequences in which one or more amino acids have been substituted with another...

...administered to an animal and the immunological response (e.g., the production of antibodies or **cell** mediated immunity) of an animal to each concentration is determined. The amounts of **antigen** administered depend on the subject, e.g. a horse or a guinea pig, the condition...

...the subject, the size of the subject, etc. Thereafter an animal so inoculated with the **antigen** can be exposed to the parasite to test the potential vaccine effect of the specific...

...other fluids or lymphocytes from the inoculated animal for cross reactivity with other closely related **Sarcocystis** spp.

A vector comprising the nucleic acids of the present invention is also provided. The vectors of the invention can be in a host capable of expressing the **antigenic** polypeptide fragments contemplated by the present invention. There are numerous E. coli expression vectors known to one of ordinary skill in the art useful for the expression of the
antigen . Other microbial hosts suitable for use include bacilli, such as
40
Bacillus subtilis, and other...

...also make expression vectors, which will typically contain expression control sequences compatible with the host **cell** (e.g., an origin of replication). In addition, any number of a variety of well...

...can be provided by insertion of a Met codon
5' and in-frame with the **antigen** . Also, the carboxyterminal extension of the **antigenic** fragments can be removed using standard oligonucleotide mutagenesis procedures.

Additionally, yeast expression can be used...
...factor leader region (encoded by the
MF.alpha.- 1 gene) is routinely used to direct **protein** secretion from yeast(Brakeetal.,1984). Theleaderregionofpre-pro-alpha-factor contains a signal peptide and a...

...sequence for a yeast protease encoded by the KEX2 gene.

this enzyme cleaves the precursor **protein** on the carboxyl side of a Lys-Arg dipeptide cleavage-signal sequence. The **antigen** coding sequence can be fused in-frame to the pre-pro-alpha-factor leader region...

...strong
transcription promoter, such as the alcohol dehydrogenase I promoter or a glycolytic promoter. The **antigen** coding sequence is followed by a translation termination codon which is followed by transcription termination signals. Alternatively, the **antigen** coding sequences can be
1 5 fused to a second **protein** coding sequence, such as Sj26 or .beta.-galactosidase, used to facilitate purification of the fusion **protein**
by affinity chromatography. The insertion of protease cleavage sites to separate the components of the fusion **protein** is applicable to constructs used for expression in yeast.

42
Mammalian cells permit the expression...

...such
as folding and cysteine pairing, addition of complex carbohydrate structures, and secretion of active **protein** . Vectors useful for the expression of **antigen** in mammalian cells are characterized by insertion of the **antigen** coding sequence between a strong viral promoter and a polyadenylation signal. The vectors can contain genes conferring either gentamicin or methotrexate resistance for use as selectable markers.

The **antigen** and immunoreactive fragment coding sequence can be

introduced into a Chinese hamster ovary **cell** line using a methotrexate resistance-encoding vector. Presence of the vector DNA in transformed cells...

...by Southern analysis and production of a cDNA or opposite strand RNA corresponding to the **antigen** coding sequence can be confirmed by northern analysis. A number of other suitable host **cell** lines capable of secreting intact proteins have been developed in the art, and include the CHO **cell** lines, HeLa cells, myeloma **cell** lines, Jurkat cells, etc. Expression vectors for these cells can include expression control sequences, such...

...The vectors containing the nucleic acid segments of interest can be transferred into the host **cell** by well-known methods, which vary depending on the type of cellular host.

For example...

...or electroporation may be used for other celluar hosts.

Alternative vectors for the expression of **antigen** in mammalian cells, those similar to those developed for the expression of human gammainterferon, tissue plasminogen activator, clotting Factor VIII, hepatitis B virus surface **antigen** , protease Nexinl, and eosinophil major basic **protein** , can be employed. Further, the vector can include CMV 1 5 promoter sequences and a...

...is also provided. The antibodies can be specifically reactive with a unique epitope of the **antigen** or they can also react with epitopes of other organisms. The term "reactive" means capable of binding or otherwise associating non randomly with an **antigen** , "Specifically reactive" as used herein refers to an antibody or other ligand that does not cross react substantially with any **antigen** other than the one specified, in this case, S. neurona. Antibodies can be made as...

...A Laborator@ Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1988). Briefly purified **antigen** can be injected into an animal in an 1 5 amount and in intervals sufficient...

...cells can be obtained from the animal. The cells are then fused with an immortal **cell** line and screened for antibody secretion. The antibodies can be used to screen clone libraries for cells secreting the **antigen** . Those positive clones can then be sequenced (see, for example, Kelly et al., 46 Bio...

...include, but are not limited to fluorescent, enzymatic and radioactive markers.

A purified S. neurona **antigen** bound to a substrate and a ligand I 0 specifically reactive with the **antigen** are also contemplated. Such a purified ligand specifically reactive with the **antigen** can be an antibody.

The antibody can be a monoclonal antibody obtained by standard methods and as described herein. The monoclonal antibody can be secreted by a hybridoma **cell** line specifically produced for that purpose
1 5 (Harlow and Lane, 1988). Likewise, nonhuman polyclonal antibodies specifically reactive with the **antigen** are within the scope of the present
invention. The polyclonal antibody can also be obtained...

...of contacting
an antibody-containing sample from the subject with a detectable mount of the **antigenic** polypeptide fragment of the present invention and detecting the reaction of the fragment and the...
...sample from the subject with
an amount of a purified antibody specifically reactive with the **antigen** as defined herein, and detecting the reaction of the ligand with the **antigen** . It is contemplated that the **antigen** will be on intact cells containing the **antigen** , or will be fragments of the **antigen** . As contemplated herein, the antibody includes any ligand which binds the **antigen** , for example, an intact antibody, a fragment of an antibody or another reagent that has reactivity with the **antigen** . The fluid sample of
this method can comprise any body fluid which would contain the **antigen** or a **cell** containing the **antigen** , such as blood, plasma, serum,
48
cerebrospinal fluid, saliva, feces and urine. Other possible examples...

...immunosorbent assays (ELISA) and
immunoblotting can be readily adapted to accomplish the detection of the **antigen** . An ELISA method effective for the detection of the **antigen** can, for example, be as follows: (1) bind the antibody to a substrate; (2) contact the bound antibody with a fluid or tissue sample containing the **antigen** ; (3) contact the above with a secondary antibody bound to a detectable moiety (e.g...
...color change. The above method can be readily modified to detect antibody as well as **antigen** .

Another immunologic technique that can be useful in the detection of S. neurona or previous...

...neurona infection utilizes
monoclonal antibodies (MAbs) for detection of antibodies specifically reactive with S. neurona **antigen** . Briefly, sera or other body fluids
49
from the subject is reacted with the **antigen** bound to a substrate (e.g. an
ELISA 96-well plate). Excess sera is thoroughly...

...labeled (enzyme-linked, fluorescent, radioactive, etc.) monoclonal antibody is then reacted with the previously reacted **antigen** serum antibody complex. The amount of inhibition of monoclonal antibody binding is measured relative to...

...neurona in a subject. Briefly, latex beads (or red blood cells) are coated with the **antigen** and mixed with a sample from the subject, such that antibodies in the tissue or body fluids that are

specifically reactive with the **antigen** crosslink with the **antigen** , causing
agglutination. The agglutinated **antigen** -antibody complexes form a precipitate, visible with the naked eye or capable of being detected...

...a
spectrophotorneter. In a modification of the above test, antibodies
50
specifically reactive with the **antigen** can be bound to the beads and **antigen** in the tissue or body fluid thereby detected.

In addition, as in a typical sandwich assay, the antibody can be bound to a substrate and reacted with the **antigen** Thereafter, a secondary labeled antibody is bound to epitopes not recognized by the first antibody and the secondary antibody is detected. Since the present invention provides S. neurona **antigen** for the detection of infectious, S.

neurona or previous S. neurona infection other serological methods...

...immunoprecipitation can also be used as
detection methods.
In the diagnostic methods taught herein, the **antigen** can be bound to a substrate and contacted by a fluid sample such as serum...

...the patient or in a partially purified form. In this manner, antibodies specific for the **antigen** (the primary antibody) will specifically react with the bound **antigen** . Thereafter, a secondary antibody bound to, or labeled with, a detectable moiety can be added...

...antibody or other ligand which is reactive, either specifically with a different epitope of the **antigen** or nonspecific ally with, the ligand or reacted antibody, will be selected for its ability...

...examples by the standard criteria applied to such selections (Harlow and Lane, 1988).

52
The **antigen** , e.g., a purified **antigenic** polypeptide fragment encoded by the Sequence Listing of this invention can be used in the construction of a **vaccine** comprising an immunogenic mount of the antigen and a pharmaceutically acceptable carrier. The vaccine can...

...can then be used in a method of preventing EPM or other complications of S. **neurona** infection.

Immunogenic amounts of the antigen can be determined using standard procedures. Briefly, various concentrations...

...The pharmaceutically acceptable carrier can comprise saline or other suitable carriers (Amon, R. (Ed.) Synthetic **Vaccines** I: 83-92, CRC Press, Inc., Boca Raton, Fla., 1987). An adjuvant can also be a part of the carrier of the **vaccine** , in which case it can be selected by
53
standard criteria based on the antigen...

...administration can
be by oral or sublingual means, or by injection, depending on the

particular **vaccine** used and the subject to whom it is administered.

It can be appreciated from the above that the **vaccine** can be used as a prophylactic or a therapeutic modality. Thus, the invention provides methods of preventing or treating S. **neurona** infection and the associated diseases by administering the **vaccine** to a subject.

Nucleic acid **vaccines** against S. **neurona** are also contemplated by the invention. The antigenic agent for use in the **vaccines** of the invention can be any nucleic acid, e.g., as set forth in the...

...to a subject. Suitable nucleic
acids include those that encode the native proteins of S. **neurona** , e.g.,
SnSAG2, SnSAG3 or SnSAG4 protein or a variant or antigenic peptide fragment thereof...

...SEQ ID NO:25 or SEQ ID NO:27.

The nucleic acid used as a **vaccine** can be e.g., a naked DNA, or the
54
nucleic acid can be incorporated...

...vector (see, e.g., Rosenberg, S. A.,
Immunity 10:281, 1999).

The presence of S. **neurona** can also be determined by detecting the presence of a nucleic acid specific for S. **neurona** or the antigens of
S. **neurona** encoded by the nucleic acids set forth herein. The present invention provides a method of detecting the presence of S. **neurona** in a subject, comprising detecting the presence of the nucleic acid encoding S. neurona. As...those
skilled in the art.

EXAMPLES
Identification and Characterization of SnSAGI
Surface biotinylation of extracellular **merozoites** revealed only
1 5 two dominant labeled molecules that migrate at about 30 kDa and...

...neurona EST database (currently
1800+ sequences) identified an orthologue of the 3 1-kDa surface **antigen** from **Sarcocystis** muris. The sequence of the S. neurona surface **antigen** gene, designated SnSAG1, is predicted to encode a
276residue **protein** with an amino-terminal signal peptide and a carboxy
61
terminal GPI anchor addition. Antiserum raised against recombinant SnSAGI recognized a 25-kDa **antigen** in western blots of non-reduced S. neurona lysates, consistent with the molecular weight predicted...

...similar to what has been observed in
western blot analyses of reduced T gondii surface **antigens** . Immunofluorescence labeling of SnSAGI during intracellular growth of S. neurona indicated that the **protein** is expressed throughout schizogony. Interestingly, a filamentous staining pattern was observed in intermediate schizonts that likely reflects localization of the surface
antigen to previously-described invaginations of the schizont surface

membrane.

Materials and Methods
1 5 Parasite culture
S. neurona strain SN3 [Granstrom, 1992 # 1 600] **merozoites** were propagated by serial passage in bovine turbinate (13T) cells and maintained in RPMI 1640...

...IO% fetal bovine
serum, 2 mM sodium pyruvate, Pen/Strep Fungizone (BioWhittaker,
62
Inc.). Extracellular **merozoites** were harvested and purified from disrupted host **cell** monolayers by filtration through 3.0 @Lrn membranes,
as described previously for Neospora caninum [Howe...
...72].

Immunoscreen ofS. neurona cDNA library
Construction and analyses of the cSn. 1 S. neurona **merozoite** cDNA library has been described previously [Howe, 2001 #1787]. The library was plaqued for 3...

...phosphate buffered
saline (PBS), 5% dry milk, 5% normal goat serum, 0.05% Tween 20.

Antigenic cDNA clones were identified by screening with cerebrospinal fluid (CSF) from a horse that had...

...the cSn. 1 filters. After washing, filters were
incubated for I hr with goat anti- **equine** IgG conjugated to horseradish peroxidase (HRP) (Jackson Immunoresearch Labs, Inc.) diluted to 1: I 0...

...neurona EST database searches and sequence analyses
S. neurona homologues to previously-characterized coccidian surface **antigens** were identified in the S. neurona clustered EST database (See, e.g., paradb.cis.upenn...
...Information (NCBI) web site (See, e.g.,
www.ncbi.nlm.nih.gov@ and the Expert **Protein** Analysis System (ExPASy) server of the Swiss Institute of Bioinformatics (See, e.g., www.expasy...

...the manufacturer's protocol (Novagen),
and monospecific polyclonal antisera. were produced against the
66
purified **protein** by immunization of a rabbit and rat (Cocalico Biologicals, Inc.).

Western blot analysis
Parasites were...

...Biotinylation of surface proteins andprecipitation with immobilized streptavidin
67
Approximately 3 x 107 freshly harvested **merozoites** were resuspended in 1 ml cold PBS (pH 7.8). Sulfo-N-hydroxy-suceinimide biotin...

...The soluble proteins were incubated

with UltraLink immobilized streptavidin (Pierce), and the precipitated biotin-labeled **protein** fraction was analyzed by western blotting, as described above.

linmunofluorescent labeling of extracellular and intracellularparasites ...

...detection of SnSAG1 on extracellular parasites and in trails deposited by gliding parasites, freshly lysed **merozoites** were suspended 68
in fresh RPMI 1640 and incubated on poly-L-lysine-coated slides...

...forrnalin-PB S containing 0. 0 1 % glutaraldehyde
For detection of SnSAG1 on intracellular parasites, **merozoites** were inoculated onto BT cells grown on LabTek chamber slides (Nunc). At 24 hr, 48...

...SnAgl.9, were chosen for further analyses.

To obtain a preliminary identification of the parasite **protein** 1 0 encoded by the selected cDNAs, the SnAgI.9 clone was used to affinity purify antibodies that bind the **antigen** expressed by this clone, and the
eluted antibodies were used to probe a western blot of S. neurona **merozoite** lysate. As shown in Fig. 1, the purified antibodies reacted with an approximately 3 1-kDa **antigen** in reduced S. neurona lysate.

1 5 Furthermore, the **antigen** revealed by the phage-purified antibodies comigrated with a **protein** that is recognized by **equine** or rabbit antisera
against S. neurona as the major immunodominant **antigen** of this parasite (Fig. 1, lanes 2 and 3). This result implies that the 22...

...during the library screen and represented by
SnAgl.8 and SnAgl.9 encode the immunodominant **antigen** of S.

neurona.

70
Full-length sequence analysis of SnAgl.8 revealed a cDNA insert of 1493 nucleotides, with an open reading frame (ORF) that encodes a 276 amino acid **protein** . Sequence analysis of SnAg1.9 indicated that this clone was virtually identical to SnAg1.8...
...160 nucleotides longer
due to an alternative polyadenylation site. A hydrophobicity plot of the encoded **protein** showed hydrophobic domains at both tennini, which correspond to a predicted signal peptide at the...

...140 Removal of the N-terminal and C
terminal signal sequences results in a mature **protein** of 242 amino acids
that has a predicted molecular weight of 24.2 kDa before...

...the
query. These searches revealed a statistically significant similarity to the 31 kDa major surface **antigen** of **Sarcocystis** muris [Eschenbacher, 1992 # 1 767] and a less significant but recognizable similarity to several SAG2-related surface **antigens** from T gondii [Lekutis, 2000

#2049].

(Fig. 2). In conjunction with the western blot analysis...

...the gene represented by the SnAgI.8 and SnAgI.9 cDNAs encodes an immunodominant surface **antigen** of S. **neurona** ; consequently, we tentatively designated this protein SnSAG1, following the genetic nomenclature that is utilized for...

...that set forth above for SnGAG1. These novel nucleotide sequences and protein 72 sequences of **Sarcocystis** **neurona** can be utilized in the production of **vaccines** and/or antigen/antibody kits for prevention and diagnosis of **Sarcocystis** **neurona** infection. One preferred embodiment of the invention is a **vaccine** comprised of an alpha virus expression vector and nucleic acid selected from the nucleic acid sequences disclosed herein.

Identification of S. **neurona** Surface Antigens and Expression as Recombinant Proteins

Analysis of the S. **neurona** EST database revealed four paralogous proteins that are homologous to the SAG and SRS surface antigens of Toxoplasma gondii. Each S. **neurona** gene was predicted to encode a **protein** that possessed an amino-terminal signal peptide and a carboxyl-tenninal glycolipid anchor site, consistent with the proteins being surface **antigens** . Because of their similarity to Toxoplasma SAGs and their probable surface display on **merozoites** , the four S. neurona proteins were designated SnSAG1, SnSAG2, SnSAG3, and SnSAG4. The four putative surface **antigens** were each expressed as a recombinant **protein** in E. coli, and these were used to immunize rabbits 73 and rats for monospecific...

...forms of native SnSAG1 and SnSAG4 are predicted to be approximately 24 kDa, but these **antigens** co-migrated at approximately 30-32 kDa and correspond to the immunodominant **antigen** Sn30 that has been described previously (See, Figure 3) (Granstrom et al., 1993; Liang et al., 1998). SnSAG1 has also been identified by others as a major surface **antigen** matching the ininumodominant Sn3O band (Ellison et al., 2002), but it is apparent that SnSAG4...

...weight. The mature form of SnSAG2 is predicted to be about 12 kDa, but this **antigen** migrated at approximately 18-19 kDa and corresponds to the previously described immunodominant Sn 1 6 **antigen** (See, Figure 3) (Granstrom et al., 1993; Liang et al., 1998). Mature SnSAG3 is predicted...

...SnSAGs under reducing conditions is a characteristic that has been observed previously for the surface **antigens** of both T gondii (Burg et al., 1988; Cesbron-Delauw et al., 1994) and N...

...recognition of recombinant SnSAG 1 (rSnSAG 1) and standard western blot analysis of complete parasite **antigen** (i.e., S. neurona **merozoite** lysate). Similar results

were
obtained with rSnSAG2, rSnSAG3, and rSnSAG4. These data
demonstrate the utility...

...S. neurona-infected horses.

Enzyme-Linked Immunosorbent Assays (ELISAs) Based
on Recombinant S. neurona Surface **Antigens** (rSnSAGs)
The rSnSAGs expressed in E. coli have been shown in western blots to
be recognized by **equine** antibodies; consequently, these recombinant
1 5 **antigens** can be utilized as the key reagents for developing ELISAs
based on single S. neum7za **antigens** . Given the teachings set forth
herein and utilizing methods known in the art, an ELISA...
...SnSA Gs.

To produce highly purified recombinant forms of the SnSAGs,
the genes for each **antigen** have been cloned into the pET22b expression
plasmid from Novagen (Madison, WI). This plasmid vector...

...ion affinity columns and allows for the
efficient one-step purification of the expressed recombinant **protein** .

Plasmid constructs were transformed into BL21 (DE3) host cells
1 0 (CodonPlus, Stratagene, Inc.), and expression of recombinant **protein**

was induced by addition of IPTG. Bacterial clones that reliably
expressed the recombinant SnSAGs were selected and cyropreserved
for future study. The recombinant S. neurona surface **antigens** have
been designated rSnSAGl, rSnSAG2, rSnSAG3, and rSnSAG4. When
1 5 recombinant **protein** is needed for use in the ELISAs, the
appropriate
bacterial clone can be grown to logarithmic phase in LB medium, and
protein expression can be induced by addition of IPTG to the culture.

The recombinant **protein** can be extracted from inclusion bodies with 6
M urea and purified from the host **cell** lysate by Ni ++ -column
chromatography according to the manufacturer's protocol (His-Bind
76
resin...

...with PBS/O. 1 % Tween 20 and incubated with horseradish
77
peroxidase (HRP)-conjugated anti- **equine** immunoglobulin secondary
antibody (Jackson Immunoresearch Labs, Inc.). The wells can again be
washed with PBS...

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Claim
... wherein the isolated nucleic acid is capable of selectively hybridizing with a nucleic acid from **Sarcocystis** neuroiza.

6 The composition of claim 1, further comprising a pharmaceutically acceptable carrier.

7 A composition comprising an isolated nucleic acid capable of encoding an **antigenic** **protein** derived from **Sarcocystis** neurona or a unique **antigenic** polypeptide fragment thereof comprised of at least a portion of a nucleotide sequence selected from...

...consisting of a E. Coli and an Alpha virus.

11 A composition comprising a purified **antigenic** polypeptide comprised of at least a portion of an amino acid sequence selected from the...

...carrier.
13 A composition comprising a purified antibody that is specifically reactive with a a **antigenic** polypeptide comprised of at least a portion of an amino acid sequence selected from the...

...NO: 30.

14 The composition of claim 13, wherein the antibody is specifically reactive with **Sarcocystis** neurona.

15 The composition of claim 13, wherein the antibody is a monospecific polyclonal antibody...

...23; SEQ ID NO: 25; SEQ ID NO:
27; SEQ ID NO: 29;
(b) an **antigenic** polypeptide comprised of at least a portion of an amino acid sequence selected from the...

...28; and SEQ ID
NO: 30; and
(c) an antibody that specifically binds to a **antigenic** polypeptide comprised of at least a portion of an amino acid sequence selected from the...
...28; and SEQ ID NO: 30.
96
. A composition for stimulating an immune response against **Sarcocystis** neurona when administered to an animal, the composition comprising an immunogenic amount of: (a) an...

...agent that can specifically stimulate an immune response against at least a portion of a **protein** or polypeptide wherein the **protein** or polypeptide is selected from the group set forth in the Sequence Listing as SEQ...

10/3,KWIC/37 (Item 8 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00849074
EQUINE **PROTOZOAL MYELOENCEPHALITIS VACCINE**
VACCIN CONTRE LA MYELOENCEPHALITE PROTOZOAIRE DU CHEVAL
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AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ
EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ
TM TR TT TZ UA UG UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW MZ SD SL SZ TZ UG ZW
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EQUINE **PROTOZOAL MYELOENCEPHALITIS VACCINE**

Fulltext Availability:
Detailed Description
Claims

English Abstract
The present invention provides an immunogenically active component comprising inactivated **Sarcocystis neurona** cells and/or inactivated Neospora hughesi cells; antigens derived therefrom; DNA derived therefrom; or a mixture; or in combination with other **vaccine** components thereof. Further provided are **vaccine** compositions useful for preventing or ameliorating **equine** protozoal myeloencephalitis infection and disease and a method for the **cell** culture propagation of protozoan parasites.

French Abstract
La presente invention concerne un composant immunogenetiquement actif comprenant des cellules inactivees de **Sarcocystis neurona** et/ou des cellules inactivees de Neospora hughesi, des antigenes derives de ces cellules, de...

...ADN derive de ces cellules, ou un melange, ou en combinaison avec d'autres composants **vaccinaux** en provenant. L'invention concerne egalement des compositions **vaccinales** permettant de prevenir ou d'ameliorer des infections et affections liees a la myeloencephalite protozoaire...

Detailed Description

EQUINE PROTOZOAL MYELOENCEPHALITIS **VACCINE**

ABSTRACT OF THE INVENTION

The present invention provides an immunogenically active component comprising inactivated **Sarcocystis neurona** cells and/or inactivated Neospora hughesi cells; antigens derived therefrom; DNA derived therefrom; or a mixture; or in combination with other **vaccine** components thereof.

Further provided are **vaccine** compositions useful for preventing or ameliorating **equine** protozoal

myeloencephalitis infection and disease and a method for the cell culture propagation of protozoan parasites.

EQUINE PROTOZOAL MYELOENCEPRALITIS **VACCINE**

BACKGROUND OF THE INVENTION

Equine protozoal myeloencephalitis (**EPM**) is a debilitating neurologic disease of **equines** which can affect the brain, the brain stem, spinal cord or any combination of these three areas of the **equine** 's central nervous system. **EPM** is caused by the protozoan parasites **Sarcocystis neurona** or Neospora hughesi.

A horse of any age, breed or gender may be af f ected by **EPM** . The disease has been reported in two-month olds, as well as thirty-year olds...

...any combination of the aforementioned signs may occur in early or less severe infections.

Initially **EPM** was thought to only be caused by **Sarcocystis** neurona. The opossum (Didelphis virginiana) has been identified as the definitive host for this agents...

...still unknown. The horse ingests feed which has been contaminated with opossum fecal material containing **Sarcocystis** neurona sporocysts. These sporocysts then excyst in the intestinal epithelium of the intermediate and incidental...

...of the intermediate host, the merezoites would encyst in the tissues of the host forming **sarcocysts** . In the case of the aberrant host, the **Sarcocystis** neurona multiply in the Central Nervous System (spinal cord) and fail to encyst. In horses, the only observed forms of **Sarcocystis** neurona have been the meront or **merozoite** .

Recently Neospora hughesi has been identified as a second organism which will cause the **EPM** clinical disease. Neospora hughesi will not only infect the spinal cord as **Sarcocystis neurona** does, but will also colonize the brain. At this point in time the definitive and...

...or water with sporulated oocysts is the route of horse infection. The oocysts will release **tachyzoites** which will infect cells as do the **merozoites** of **Sarcocystis neurona** .

In both cases the horse is an aberrant dead-end host and infectious forms of...

...from an infected horse to a definitive or true intermediate host.

There is currently no **vaccine** or approved animal drug product available for the effective treatment of

EPM . The currently available treatments are expensive, of limited efficacy and may include adverse side effects such as anemia, abortion, diarrhea, low white blood **cell** counts or the like. There remains an unfulfilled need for treatment for **EPM** -afflicted **equines** , particularly horses, which is effective, convenient to administer and useful for the reduction of resistant...

...this invention to provide an immunogenically active component useful for the prevention or amelioration of **EPM** .

It is another object of this invention to provide a **vaccine** composition suitable for use in **equines** against infection and disease caused by the protozoan parasites **Sarcocystis neurona** and/or iVeospora hughesi. It is a further object of this invention to provide a method for the prevention or amelioration of **EPM** disease in **equines** that need such protection. Other objects and features of the invention will become apparent from...

...SUMMARY OF THE INVENTION
The present invention provides an immunogenically active component which comprises inactivated **Sarcocystis neurona** cells or inactivated Neospora hughesi cells; DNA derived therefrom; or a mixture; or in combination with other **vaccine** components.

The present invention further provides an immunogenically active component which comprises a member selected from the group consisting of merozoite antibody inducing, inactivated **Sarcocystis neurona** cells; tachyzoite antibody inducing, inactivated Neospora hughesi cells; a merozoite or tachyzoite antibody inducing antigen...

...a merozoite or tachyzoite antibody immune response; and a mixture thereof.

Further provided is a **vaccine** composition which comprises an effective immunizing amount of at least one of the above-said immunogenically active components and a pharmacologically acceptable carrier.

Still further provided is a **vaccine** composition which comprises a) an effective amount of one immunologically active component selected from merozoite antibody inducing, inactivated **Sarcocystis neurona** cells; a **merozoite** antibody inducing **antigen** derived or extracted from said cells; DNA derived from said cells capable of inducing a **merozoite** antibody immune response, and a mixture thereof; b) an effective amount of a second immunologically active component selected from **tachyzoite** antibody inducing, inactivated Neospora hughesi cells; a **tachyzoite** antibody inducing **antigen** derived or extracted from said cells; DNA derived from said cells capable of

inducing a **tachyzoite** antibody immune response; and a mixture thereof; and c) a pharmacologically acceptable carrier.

The present...

...also provides a method for the prevention or amelioration of infection or disease caused by **Sarcocystis** neurona protozoa in **equines** that need such protection. The method for the prevention or amelioration of **EPM** infection or disease in **equines** comprises administering to said **equine** an immunogenically active component which comprises a member selected from the group consisting of **merozoite** antibody inducing, inactivated **Sarcocystis** neurona cells; **tachyzoite** antibody inducing, inactivated lVeospora hughesi cells; a **merozoite** or **tachyzoite** antibody inducing **antigen** derived from said cells; DNA derived from said cells capable of inducing a **merozoite** or **tachyzoite** antibody immune response; or a mixture thereof; and, optionally, a pharmacologically acceptable carrier.

Also provided is a method for the **cell** culture propagation of protozoan parasites, including **Sarcocystis** spp. and Neospora spp.

DETAILED DESCRIPTION OF THE INVENTION

Sa.rcoc.ystis neurona or Neospo-ra hughesi protozoa are the causative agents of **equine** protozoal myeloencephalitis (**EPM**) disease, which is a serious, and sometimes fatal, neurological disease in **equines** , particularly horses. **EPM** symptoms include hypermetria, decreased proprioception, weakness, cranial nerve deficits, general ataxia or the like. The...

...as the definitive host for these organisms. However an intermediate host is, as yet, unknown. **Equines** are the aberrant host and apparently become infected when ingesting feed which has been contaminated with the **Sarcocystis** **neurona** or Neospora hughesi protozoans via opossum fecal contamination. **EPM** disease when untreated will progress from initial numbness of limbs to final central nervous system destruction, resulting in death. Heretofore, there were no known **vaccination** or immunization treatments available against **EPM** .

Surprisingly, it has now been found that an immunogenically active component which comprises inactivated **Sarcocystis** **neurona** cells or antigens, subunit proteins or plasmid DNA; inactivated Neospora 5 hughesi cells or antigens...

...proteins or plasmid DNA; or mixtures thereof may be administered in the form of a **vaccine** composition to prevent or ameliorate **EPM** disease in **equines** , particularly horses. Antigens derived from **Sarcocystis** **neurona** or Neospora hughesi may be

obtained using conventional procedures such as outer membrane extraction. Plasmid DNA derived from Sarcoc.ystis **neurona** or Neospora hughesi may be obtained via isolation from sources such as the fluids or tissues of **equine** mammalians diagnosed to have **EPM** . Such sources include cerebral spinal fluid or sections of spinal cord or brain. Alternatively, the...

...from
feces or intestinal scrapings of opossums or other wild life present in endemic locales. **Sarcocystis** Spp. or Meospora SPP. cells, thus obtained, may be maintained in the infected **equine** or in suitable tissue culture media, such as RPMI 1640 medium or in cells known in the art such as African green monkey kidney (Vero) cells or **equine** dermal (E. Derm) cells. The **Sarcocystis** Spp. or Neospora Spp. protozoa may then be separated from the tissue culture of **cell** media using conventional techniques such as centrifugation, filtration, or the like. A useful starting isolate for the **vaccines** of the invention include, for example, for **Sarcocystis** **neurona** , the isolate designated SN3; other such isolates are those known as SN1, SN2, SN4, SN5...

...Davis, Oregon State University, the University of Missouri and others. A culture of one such **Sarcocystis** **neurona** isolate designated SN9, originally isolated from the intestinal scrapings of the opossum and confirmed to be a representative **Sarcocystis** **neurona** by PCR, was deposited with the ATCC on January 25, 2001, and given ATCC Accession No. PTA A useful starting isolate for the **vaccines** of the invention include, for example, for Neospora hughesi, the isolate designated NEQ1; another such...

...US 6,071,737. Surprisingly, it has now been found that protozoan parasites such as **Sarcocystis** spp.

or Neospora Spp. may be propagated in increased yield and increased active viability via **cell** culture propagation by growing suitable cells to a monolayer having a confluency of about 80...

...decanting
the growth media; refeeding the cells with fresh growth media; inoculating the cells with **merozoites** or **tachyzoites** ; after 4-12 days, decanting the growth media; and refeeding the inoculated cells a second...

...with 10% fetal bovine
serum, iron fortified fetal calf serum or donor serum.

When the **cell** monolayer has been formed, the culture is decanted to remove the original growth media, the...
...up to 10% fetal
bovine serum. The refed cells are then inoculated with merczoites or **tachyzoites** , held for 4 to 12 days and decanted to remove the growth media. The culture...

...of
cytopathology of >60% is obtained, the culture may be harvested.

The thus-obtained whole **cell** isolates of **Sarcocystis** Spp. or Neospora Spp. protozoa may be inactivated by conventional inactivating means, for example chemical...

...gluteraldehyde, sodium dodecyl sulfate, or the like or a mixture thereof, preferably formalin. Said whole **cell** isolates may also be inactivated by heat or psoralen in the presence of ultraviolet light...

...i.e., to stimulate the production of antibodies, particularly humoral antibodies , or to stimulate a **cell** mediated response. For example, the ability to stimulate the production of circulating or secretory antibodies or the production of a **cell** -mediated response in local mucosal ...evoke an immune response.

Amounts wherein the dosage unit comprises at least about X104 inactivated **Sarcocystis** Spp. cells or Neospora Spp.

cells or a mixture thereof, preferably at least about 1X106...

...also contain other active components such as an antipathogenic component directed against rabies virus, Eastern **equine** encephalitis virus, Western **equine** encephalitis virus, Venezuelan **equine** encephalitis virus, **equine** herpes virus such as EHV-1 or EHV-4, Ehrlichia risticii, Streptococcus equi, tetanus toxoid...

...intranasally.

The vaccine composition of the invention is useful for the prevention or amelioration of **EPM** infections in **equine** that need such protection. In actual practice, the vaccine composition of the invention is administered...

...effective amounts according to a schedule determined by the time of potential exposure to infective **Sarcocystis** Spp. or Neospora Spp. sporocysts. In this way, the treated animal may have time to...

...the appended claims.

Unless otherwise noted, all parts are parts by weight.

EXAMPLE 1
A - **Vaccine** preparation
An **equine** spinal cord isolate of **Sarcocystis neurona** is obtained from a horse which has been diagnosed to have **EPM** . The isolate is cultivated in multiple cultures of E. Derm cells in RPMI tissue culture medium at 37'C.

These **merozoite** harvests are counted at the time of harvest and then inactivated by means of addition...

...concentrated against 0.01M phosphate buffered saline to a level of 3.14xl 0 7 **merozoites** per mL.

The vaccines are formulated by suspending the appropriate volume of inactivated cells in...a final dilution of 1:10,000.

EXAMPLE 2

Antibody response to intramuscular inlection of **vaccine**

In this evaluation, horses that are found to be naive to Sarcocystis neurona merozoite antigen...

...level of 1x105 merozoites per dose; a second group of twenty-one horses are administered **vaccine** blended at 1X106 merozoites per dose; a third group of ten horses are administered **vaccine** at 1X107 merozoites per dose; and a fourth of group of ten horses are maintained as non **vaccinated** environmental controls. Treated horses are given a first dose of **vaccine** according to the group to which they are assigned. At twenty-one days following administration of the first dose, a second dose of the same **vaccine** is administered. All horses are bled for serum at the time of administration of the...

...and at weekly intervals through 28 days post second dose administration.

In this evaluation, the **vaccine** compositions contain formalin-inactivated, E. Derm cell line-grown Sarcoc vstis **neurona** merozoites with an adjuvant system. The method of serologic measurement of antibodies is conducted by IFA. The IFA is run using Vero cell line-grown Sarcocystis **neurona** merozoites to eliminate anti-E. Derm antibody titers.

The serological data is shown in Table I below, wherein: 0 DPV 1 designates day zero, pre **vaccination** ; 0 DPV 2 designates day zero, post **vaccination** ; 7 DPV 2 designates day 7, post **vaccination** ; and 14 DPV 2 designates day 14, post **vaccination** .

As can be seen from the data on Table I, treated horses from all groups...

...low to non-existent antibody level. The level of response in the horses that received **vaccine** was dependent upon the level of antigen in the **vaccine** that they received.

Table I

EPM (Sarcocystis **neurona**) Dose Titration IFA Serolog

No, **Vaccine** **Antigen** Load 0 DPV 1 0 DPV 2 7 DPV 2 14 DPV 2

1 lxlO...

...10

caninum by use of a modified agglutination test with formalin-preserved tachyzoites and mercaptoethanol. RESULTS: Antibodies against S **neurona** and T gondii were detected in 36 and 16 of 101 horses, respectively. Cross-reactivity between antibodies against T gondii and S **neurona** was not detected. Antibodies against N caninum were not detected in any samples. CONCLUSIONS AND CLINICAL RELEVANCE: The high prevalence of antibodies against S **neurona** detected in clinically normal horses emphasizes the importance of examining CSF for antibodies when establishing a diagnosis of equine protozoal myeloencephalitis.

Tags: Female; Male

Descriptors: *Coccidiosis--veterinary--VE; *Horse Diseases--epidemiology --EP; *Neospora--immunology--IM; *Sarcocystosis--veterinary--VE; *Toxoplasm osis, Animal--epidemiology--EP; Animals; Antibodies, Protozoan--blood--BL; Brazil--epidemiology--EP; Coccidiosis--epidemiology--EP; Horses; **Sarcocyst is** --immunology--IM; Sarcocystosis--epidemiology--EP; Seroepidemiologic Studies; Toxoplasma--immunology--IM

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 19991130

Record Date Completed: 19991130

5/9/2

DIALOG(R)File 155:MEDLINE(R)

13518527 PMID: 10489203

Prevalence of antibodies to Sarcocystis neurona , **Toxoplasma gondii and Neospora caninum in horses from Argentina.**

Dubey J P; Venturini M C; Venturini L; McKinney J; Pecoraro M

Parasite Biology and Epidemiology Laboratory, United States Department of Agriculture, Agricultural Research Service, Livestock and Poultry Sciences Institute, Beltsville, MD 20705-2350, USA. jdubey@lpsi.barc.usda.gov

Veterinary parasitology (NETHERLANDS) Sep 15 1999, 86 (1) p59-62, ISSN 0304-4017 Journal Code: 7602745

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Sera from 76 horses from Argentina were examined for antibodies to **Sarcocystis neurona** , Toxoplasma gondii and Neospora caninum. Antibodies to S. **neurona** were found in 27 (35.5%) of 76 horses using immunoblots with culture derived merozoites as **antigen** . Antibodies to T. gondii were found in 10 (13.1%) of 76 horses by using the modified agglutination test with formalin-fixed tachyzoites and mercaptoethanol; titers were 1:25 (two horses), 1:50 (six horses), 1:100 (two horses), and 1:200 (one horse). Antibodies to N. caninum were not found in any of the 76 horses by the use of N. caninum agglutination test. This is the first report of S. **neurona** infection in horses in Argentina.

Descriptors: *Coccidiosis--veterinary--VE; *Horse Diseases--epidemiology --EP; *Neospora--immunology--IM; * **Sarcocystis** --immunology--IM; *Toxoplasma--immunology--IM; Agglutination Tests--veterinary--VE; Animals; Antibodies, Protozoan--blood--BL; Argentina--epidemiology--EP; Blotting, Western--veterinary--VE; Coccidiosis--epidemiology--EP; Horse Diseases --parasitology--PS; Horses; Neospora--isolation and purification--IP; **Sarcocystis** --isolation and purification--IP; Sarcocystosis--epidemiology --EP; Sarcocystosis--veterinary--VE; Seroepidemiologic Studies; Toxoplasma

```
? e e3

Ref    Items Type  RT  Index-term
R1      1390        3 *SARCOCYSTIS
R2      1050   X       DC=B1.500.841.75.189.250.750.750. (SARCOCYSTIS)
R3        90   X    1  SARCOSPORIDIA
R4       104   B    5  SARCOCYSTIDAE
? s r1-r5
            1390  SARCOCYSTIS
            1050  DC=B1.500.841.75.189.250.750.750.  (SARCOCYSTIS)
              90  SARCOSPORIDIA
             104  SARCOCYSTIDAE
               0
      S1    1448  R1-R5
? s s1 and neurona?
            1448  S1
          124183  NEURONA?
      S2     192  S1 AND NEURONA?
? s s2/2001:2005
             192  S2
         2479289  PY=2001 : PY=2005
      S3     120  S2/2001:2005
? s s2 not s3
             192  S2
             120  S3
      S4      72  S2 NOT S3
? s s4 and (antigen? or protein? or vaccin? or immuni?)
              72  S4
          604732  ANTIGEN?
         1810838  PROTEIN?
          157100  VACCIN?
          212335  IMMUNI?
      S5      15  S4 AND (ANTIGEN? OR PROTEIN? OR VACCIN? OR IMMUNI?)
? t s5/9/all

 5/9/1
DIALOG(R)File 155:MEDLINE(R)
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13541431   PMID: 10511862
   Serologic prevalence of Sarcocystis neurona , Toxoplasma gondii, and
Neospora caninum in horses in Brazil.
  Dubey J P; Kerber C E; Granstrom D E
  Parasite Biology and Epidemiology Laboratory, United States Department of
Agriculture, Beltsville Agricultural Research Center, MD 20705-2350, USA.
  Journal of the American Veterinary Medical Association (UNITED STATES)
Oct 1 1999,  215  (7)  p970-2,  ISSN 0003-1488   Journal Code: 7503067
  Publishing Model Print
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: MEDLINE; Completed
  Subfile:   INDEX MEDICUS
  OBJECTIVE:  To determine serologic prevalence of Sarcocystis neurona ,
Toxoplasma gondii, and Neospora caninum in horses in Brazil. DESIGN:
Prevalence survey. ANIMALS: 101 Thoroughbreds in Brazil. PROCEDURE: Blood
samples were obtained from horses and tested for serum antibodies against S
 neurona by use of an immunoblot procedure with culture-derived S neurona
 merozoites as antigen , and for serum antibodies against T gondii and N
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Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

We report a simple, economical, and efficient protocol for **protein** purification from cells. First, **proteins** of cell lysates were separated by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted to **protein** -blotting membrane. The blots were stained with Coomassie blue or developed by immunoblotting to visualize specific **proteins** . The bands corresponding to those visible by immunoblotting were excised from the dye-stained blots and subjected to isoelectric focusing. The focused gel was stained with Coomassie blue. Finally, the stained bands were excised and subjected to another SDS-PAGE separation and electrotransferred back to **protein** -blotting membrane. At this stage, the purified **proteins** were suitable for microsequencing. We have tested the feasibility of this novel technique by purifying **proteins** with molecular weights ranging from 19 to 100 kDa from a lysate of **Sarcocystis neurona** , the etiologic agent of equine protozoal myeloencephalitis. The purity of **proteins** was demonstrated by reverse-phase high-performance liquid chromatography. Partial sequences of these purified **proteins** were obtained by N-terminal or digestive sequencing.

Tags: Research Support, Non-U.S. Gov't
Descriptors: ***Antigen** s, Protozoan--isolation and purification--IP; ***Proteins** --isolation and purification--IP; Animals; Chromatography, High Pressure Liquid; Electrophoresis, Polyacrylamide Gel; Immunoblotting; Isoelectric Focusing; Membranes, Artificial; **Sarcocystis** --immunology--IM
CAS Registry No.: 0 (Antigens, Protozoan); 0 (Proteins)
Record Date Created: 19970904
Record Date Completed: 19970904

5/9/13
DIALOG(R)File 155:MEDLINE(R)

11633290 PMID: 8944807
Neosporosis as a cause of equine protozoal myeloencephalitis.
Marsh A E; Barr B C; Madigan J; Lakritz J; Nordhausen R; Conrad P A
Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis 95616-8745, USA.
Journal of the American Veterinary Medical Association (UNITED STATES) Dec 1 1996, 209 (11) p1907-13, ISSN 0003-1488 Journal Code: 7503067
Publishing Model Print
Document type: Case Reports; Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Neosporosis was diagnosed in an 11-year-old Quarter Horse gelding with clinical signs and diagnostic test results compatible with equine protozoal myeloencephalitis (EPM). Presumptive postmortem diagnosis of EPM attributable to **Sarcocystis neurona** infection is generally made on the basis of detecting an antibody titer to S **neurona** in the CSF or characteristic histologic lesions, even when parasites have not been specifically identified. Neosporosis was confirmed in the horse described here by use of immunohistochemical examination, in vitro culturing, and

--veterinary--VE; Animals; Antibodies, Protozoan--blood--BL; Antibodies, Protozoan--cerebrospinal fluid--CF; Blotting, Western--veterinary--VE; Encephalomyelitis--blood--BL; Encephalomyelitis--cerebrospinal fluid--CF; Encephalomyelitis--parasitology--PS; Enzyme-Linked Immunosorbent Assay --veterinary--VE; Equidae; Horses; Injections, Spinal--veterinary--VE; Sarcocystosis--blood--BL; Sarcocystosis--cerebrospinal fluid--CF; Sarcocystosis--parasitology--PS

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20001103

Record Date Completed: 20001103

5/9/7

DIALOG(R)File 155:MEDLINE(R)

12758328 PMID: 10690772

Improvement of western blot test specificity for detecting equine serum antibodies to Sarcocystis neurona .

Rossano M G; Mansfield L S; Kaneene J B; Murphy A J; Brown C M; Schott H C; Fox J C

Animal Health Diagnostic Laboratory, The Population Medicine Center, Michigan State University, East Lansing 48824, USA.

Journal of veterinary diagnostic investigation - official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc (UNITED STATES) Jan 2000, 12 (1) p28-32, ISSN 1040-6387

Journal Code: 9011490

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

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Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Equine protozoal myeloencephalitis (EPM) is a neurological disease of horses and ponies caused by the apicomplexan protozoan parasite **Sarcocystis neurona** . The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and to assess the ability of bovine antibodies to **Sarcocystis** cruzi to act as a blocking agent to minimize false-positive results in the western blot test for S. **neurona** . **Sarcocystis neurona** merozoites harvested from equine dermal cell culture were heat denatured, and the **proteins** were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient gel. Separated **proteins** were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6 horses with S. **neurona** infections (confirmed by culture from neural tissue) and 57 horses without infections (horses from the Eastern Hemisphere, where S. **neurona** does not exist) were tested by Western blot. Horses from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-kD bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the equine samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16-kD bands ($P<0.001$, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking **proteins** not specific to S. **neurona** and using reactivity to the 30- and 16-kD bands as the criterion for a

ultrastructural and molecular characterization of parasites from infected tissues. Antibody testing of serum and CSF samples indicated that Neospora-specific anti-bodies can react with S **neurona** **proteins** on western blot analysis. The confirmation that neosporosis in horses can mimic EPM emphasizes the need to broaden the etiologic definition of EPM beyond infections exclusively attributable to S **neurona** .

Tags: Male; Research Support, Non-U.S. Gov't

Descriptors: *Coccidiosis--veterinary--VE; *Encephalomyelitis--veterinary --VE; *Horse Diseases--parasitology--PS; *Neospora --isolation and purification--IP; Animals; Antibodies, Protozoan--cerebrospinal fluid--CF; Antibodies, Protozoan--immunology--IM; **Antigens** , Protozoan--analysis--AN ; Coccidiosis--parasitology--PS; Encephalomyelitis--parasitology--PS; Horses; Immunohistochemistry; Neospora--immunology--IM; Neospora --ultrastructure--UL; Spinal Cord--parasitology--PS; Spinal Cord --ultrastructure--UL

CAS Registry No.: 0 (Antibodies, Protozoan); 0 (Antigens, Protozoan)

Record Date Created: 19970130

Record Date Completed: 19970130

5/9/14

DIALOG(R)File 155:MEDLINE(R)

10136275 PMID: 8466988

Equine protozoal myeloencephalitis: antigen **analysis of cultured** Sarcocystis neurona **merozoites.**

Granstrom D E; Dubey J P; Davis S W; Fayer R; Fox J C; Poonacha K B; Giles R C; Comer P F

Department of Veterinary Science, University of Kentucky, Lexington 40546-0099.

Journal of veterinary diagnostic investigation - official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc (UNITED STATES) Jan 1993, 5 (1) p88-90, ISSN 1040-6387

Journal Code: 9011490

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

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Subfile: INDEX MEDICUS

Antigens of cultured **Sarcocystis** **neurona** merozoites were examined using immunoblot analysis. Blotted **proteins** were probed with S. cruzi, S. muris, and S. **neurona** antisera produced in rabbits, S. fayeri (pre- and post-infection) and S. **neurona** (pre- and post-inoculation) sera produced in horses, immune sera from 7 histologically confirmed cases of equine protozoal myeloencephalitis (EPM), and pre-suckle serum from a newborn foal. Eight **proteins** , 70, 24, 23.5, 22.5, 13, 11, 10.5, and 10 Kd, were detected only by S. **neurona** antiserum and/or immune serum from EPM-affected horses. Equine sera were titered by the indirect immunofluorescent antibody (IFA) method using air-dried, cultured S. **neurona** merozoites. Anti- **Sarcocystis** IFA titers were found in horses with or without EPM. Serum titers did not correspond to the number of specific bands recognized on immunoblots.

Tags: Research Support, Non-U.S. Gov't

Descriptors: ***Antigen** s, Protozoan--analysis--AN; *Horse Diseases; * **Sarcocystis** --immunology--IM; *Sarcocystosis--veterinary--VE; Animals; **Antigens** , Protozoan--isolation and purification--IP; Cattle; Cells,

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*File 467: F467 no longer updates; see Help News467. 7.

Set	Items	Description

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Set	Items	Description
S1	1448	R1-R5
S2	192	S1 AND NEURONA?
S3	120	S2/2001:2005
S4	72	S2 NOT S3
S5	15	S4 AND (ANTIGEN? OR PROTEIN? OR VACCIN? OR IMMUNI?)

? t s5/9/7 8 12 13 14 15

5/9/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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12758328 PMID: 10690772
Improvement of western blot test specificity for detecting equine serum antibodies to Sarcocystis neurona .
Rossano M G; Mansfield L S; Kaneene J B; Murphy A J; Brown C M; Schott H C; Fox J C
Animal Health Diagnostic Laboratory, The Population Medicine Center, Michigan State University, East Lansing 48824, USA.
Journal of veterinary diagnostic investigation - official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc (UNITED STATES) Jan 2000, 12 (1) p28-32, ISSN 1040-6387
Journal Code: 9011490
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Equine protozoal myeloencephalitis (EPM) is a neurological disease of horses and ponies caused by the apicomplexan protozoan parasite **Sarcocystis neurona** . The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and

were stained with Coomassie blue or developed by immunoblotting to visualize specific **proteins** . The bands corresponding to those visible by immunoblotting were excised from the dye-stained blots and subjected to isoelectric focusing. The focused gel was stained with Coomassie blue. Finally, the stained bands were excised and subjected to another SDS-PAGE separation and electrotransferred back to **protein** -blotting membrane. At this stage, the purified **proteins** were suitable for microsequencing. We have tested the feasibility of this novel technique by purifying **proteins** with molecular weights ranging from 19 to 100 kDa from a lysate of **Sarcocystis neurona** , the etiologic agent of equine protozoal myeloencephalitis. The purity of **proteins** was demonstrated by reverse-phase high-performance liquid chromatography. Partial sequences of these purified **proteins** were obtained by N-terminal or digestive sequencing.

Tags: Research Support, Non-U.S. Gov't

Descriptors: ***Antigen** s, Protozoan--isolation and purification--IP; ***Proteins** --isolation and purification--IP; Animals; Chromatography, High Pressure Liquid; Electrophoresis, Polyacrylamide Gel; Immunoblotting; Isoelectric Focusing; Membranes, Artificial; **Sarcocystis** --immunology--IM

CAS Registry No.: 0 (Antigens, Protozoan); 0 (Proteins)

Record Date Created: 19970904

Record Date Completed: 19970904

175904 Subscriber

5/9/13 (Item 13 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

~~11~~633290 PMID: 8944807

Neosporosis as a cause of equine protozoal myeloencephalitis.

Marsh A E; Barr B C; Madigan J; Lakritz J; Nordhausen R; Conrad P A

Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis 95616-8745, USA.

Journal of the American Veterinary Medical Association (UNITED STATES) Dec 1 1996, 209 (11) p1907-13, ISSN 0003-1488 Journal Code: 7503067

Publishing Model Print

Document type: Case Reports; Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Neosporosis was diagnosed in an 11-year-old Quarter Horse gelding with clinical signs and diagnostic test results compatible with equine protozoal myeloencephalitis (EPM). Presumptive postmortem diagnosis of EPM attributable to **Sarcocystis neurona** infection is generally made on the basis of detecting an antibody titer to S **neurona** in the CSF or characteristic histologic lesions, even when parasites have not been specifically identified. Neosporosis was confirmed in the horse described here by use of immunohistochemical examination, in vitro culturing, and ultrastructural and molecular characterization of parasites from infected tissues. Antibody testing of serum and CSF samples indicated that Neospora-specific anti-bodies can react with S **neurona** **proteins** on western blot analysis. The confirmation that neosporosis in horses can mimic EPM emphasizes the need to broaden the etiologic definition of EPM beyond infections exclusively attributable to S **neurona** .

Tags: Male; Research Support, Non-U.S. Gov't

Descriptors: *Coccidiosis--veterinary--VE; *Encephalomyelitis--veterinary --VE; *Horse Diseases--parasitology--PS; *Neospora --isolation and

12992935 PMID: 10946139

Inoculation of Sarcocystis **neurona** merozoites **into the central nervous system of horses.**

Lindsay D S; Dykstra C C; Williams A; Spencer J A; Lenz S D; Palma K; Dubey J P; Blagburn B L

Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, 24061-0342, USA. lindsayd@vt.edu

Veterinary parasitology (NETHERLANDS) Sep 20 2000, 92 (2) p157-63, ISSN 0304-4017 Journal Code: 7602745

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Equine protozoal myeloencephalitis (**EPM**) is a neurologic syndrome in horses from the Americas and is usually caused by infection with the apicomplexan parasite, **Sarcocystis neurona** . A horse model of **EPM** is needed to test the efficacy of chemotherapeutic agents and potential **vaccines** . Five horses that were negative for antibodies to S. **neurona** in their serum and cerebrospinal fluid (CSF) were injected in the subarachnoid space with living **merozoites** of the SN2 isolate of S. **neurona** . None of the horses developed clinical disease or died over a 132-day observation period. All five horses developed antibodies to S. **neurona** in their CSF and serum 3-4 weeks after injection. Two of the horses were examined at necropsy and no parasite induced lesions were observed in their tissues and no parasites were recovered from portions of their spinal cords inoculated on to **cell** cultures. Results of this study demonstrate that **merozoites** of the SN2 isolate of S. neurona will induce seroconversion but not clinical disease when inoculated directly into the CSF of nonimmune horses.

Tags: Female; Male; Research Support, Non-U.S. Gov't

Descriptors: *Encephalomyelitis--veterinary--VE; *Horse Diseases --parasitology--PS; * **Sarcocystis** --pathogenicity--PY; * **Sarcocystosis** --veterinary--VE; Animals; Antibodies, Protozoan--blood--BL; Antibodies, Protozoan--cerebrospinal fluid--CF; Blotting, Western--veterinary--VE; Encephalomyelitis--blood--BL; Encephalomyelitis--cerebrospinal fluid--CF; Encephalomyelitis--parasitology--PS; Enzyme-Linked Immunosorbent Assay --veterinary--VE; Equidae; Horses; Injections, Spinal--veterinary--VE; **Sarcocystosis** --blood--BL; **Sarcocystosis** --cerebrospinal fluid--CF; **Sarcocystosis** --parasitology--PS

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20001103

Record Date Completed: 20001103

10/9/59 (Item 1 from file: 129)

DIALOG(R)File 129:PHIND(Archival)

00731140

Fort Dodge's EPM **(equine protozoal myeloencephalitis) vaccine licence renewed:**

Animal-Pharm 480 p20, November 02, 2001 (20011102)

STORY TYPE: B WORD COUNT: 174

The US Department of Agriculture (USDA)'s Center for Veterinary

to assess the ability of bovine antibodies to **Sarcocystis** cruzi to act as a blocking agent to minimize false-positive results in the western blot test for S. **neurona** . **Sarcocystis** **neurona** merozoites harvested from equine dermal cell culture were heat denatured, and the **proteins** were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient gel. Separated **proteins** were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6 horses with S. **neurona** infections (confirmed by culture from neural tissue) and 57 horses without infections (horses from the Eastern Hemisphere, where S. **neurona** does not exist) were tested by Western blot. Horses from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-kD bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the equine samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16-kD bands ($P<0.001$, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking **proteins** not specific to S. **neurona** and using reactivity to the 30- and 16-kD bands as the criterion for a positive test.

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Antibodies, Protozoan--analysis--AN; *Cattle Diseases --parasitology--PS; *Encephalomyelitis, Equine--virology--VI; * **Sarcocystis** --immunology--IM; *Sarcocystosis--veterinary--VE; Animals; Blotting, Western--standards--ST; Cattle; Cattle Diseases--genetics--GE; Cattle Diseases--immunology--IM; Encephalomyelitis, Equine--genetics--GE; Encephalomyelitis, Equine--immunology--IM; **Sarcocystis** --genetics--GE; Sarcocystosis--genetics--GE; Sarcocystosis--immunology--IM; Sensitivity and Specificity

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20000321

Record Date Completed: 20000321

5/9/8 (Item 8 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

12685232 PMID: 10608440

Prevalence of antibodies to Neospora sp. in horses from Alabama and characterisation of an isolate recovered from a naturally infected horse [corrected]

Cheadle M A; Lindsay D S; Rowe S; Dykstra C C; Williams M A; Spencer J A; Toivio-Kinnucan M A; Lenz S D; Newton J C; Rolsma M D; Blagburn B L

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849, USA.

International journal for parasitology (ENGLAND) Oct 1999, 29 (10) p1537-43, ISSN 0020-7519 Journal Code: 0314024

Publishing Model Print; Erratum in Int J Parasitol 2000 Apr 24;30(5) 677

Document type: Case Reports; Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

An IFAT was used to determine the prevalence of Neospora-specific IgG antibodies in serum from Alabama horses. Serum samples (n = 536) were from

positive test.
Tags: Research Support, Non-U.S. Gov't
Descriptors: *Antibodies, Protozoan--analysis--AN; *Cattle Diseases --parasitology--PS; *Encephalomyelitis, Equine--virology--VI; * **Sarcocystis** --immunology--IM; *Sarcocystosis--veterinary--VE; Animals; Blotting, Western--standards--ST; Cattle; Cattle Diseases--genetics--GE; Cattle Diseases--immunology--IM; Encephalomyelitis, Equine--genetics--GE; Encephalomyelitis, Equine--immunology--IM; **Sarcocystis** --genetics--GE; Sarcocystosis--genetics--GE; Sarcocystosis--immunology--IM; Sensitivity and Specificity
CAS Registry No.: 0 (Antibodies, Protozoan)
Record Date Created: 20000321
Record Date Completed: 20000321

5/9/8
DIALOG(R)File 155:MEDLINE(R)

12685232 PMID: 10608440
Prevalence of antibodies to Neospora sp. in horses from Alabama and characterisation of an isolate recovered from a naturally infected horse [corrected]
Cheadle M A; Lindsay D S; Rowe S; Dykstra C C; Williams M A; Spencer J A; Toivio-Kinnucan M A; Lenz S D; Newton J C; Rolsma M D; Blagburn B L
Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849, USA.
International journal for parasitology (ENGLAND) Oct 1999, 29 (10) p1537-43, ISSN 0020-7519 Journal Code: 0314024
Publishing Model Print; Erratum in Int J Parasitol 2000 Apr 24;30(5) 677
Document type: Case Reports; Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
An IFAT was used to determine the prevalence of Neospora-specific IgG antibodies in serum from Alabama horses. Serum samples (n = 536) were from asymptomatic horses routinely submitted for equine infectious anaemia virus infection testing. We also subjected a 13-year-old horse with CNS disease to necropsy examination for isolation and in vitro cultivation of protozoal organisms. In antemortem tests, this horse was positive for antibodies to Neospora sp. in the IFAT and western immunoblot. Results of the prevalence survey indicated that IgG antibodies to Neospora were present in 62 (11.5%) of the 536 serum samples. Endpoint titres for the positive samples were 1:50 (35/6.5%), 1:100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). Tachyzoites were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the horse with CNS disease. Tachyzoites reacted with known N. caninum-positive serum from horses, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or equine anti- **Sarcocystis neurona** serum. Ultrastructural features of tachyzoites and results of comparison of tachyzoite immunodominant **proteins** revealed that they were identical to those of N. hughesi, a species described recently from a naturally infected horse. The isolate recovered from the naturally infected horse in the present study (designated NA1) is thought to be an isolate of N. hughesi, although confirmation of this awaits additional molecular characterisation. These results provide some additional evidence that N. hughesi is a valid species and that Neospora infections in horses may occur in widely separated geographic regions of the United States.

C) inoculating said cells with **merozoites** or **tachyzoites** ;
d) holding the inoculated cells for 4-12 days;
e) decanting the supplemented growth media...

1/3,KWIC/25 (Item 1 from file: 10)
DIALOG(R)File 10:AGRICOLA

3957097 22039908 Holding Library: AGL
Prevalence of antibodies to Neospora sp. in horses from Alabama and characterisation of an isolate recovered from a naturally infected horse. [Erratum: Apr 24, 2000, v. 30 (5), p. 677.]
Cheadle, M.A. Lindsay, D.S.; Rowe, S.; Dykstra, C.C.; Williams, M.A.; Spencer, J.A.; Toivio-Kinnucan, M.A.; Lenz, S.D.; Newton, J.C.; Rolsma, M.D.; Blagburn, B.L.
Auburn University, AL.
Oxford : Elsevier Science Ltd.
International journal for parasitology Oct 1999. v. 29 (10) p. 1537-1543.
ISSN: 0020-7519 CODEN: IJPYBT
DNAL CALL NO: QH547.I55
Language: English

...100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). **Tachyzoites** were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the horse with CNS disease. **Tachyzoites** reacted with known N. caninum-positive serum from horses, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or equine anti-Sarcocystis **neurona** serum. Ultrastructural features of **tachyzoites** and results of comparison of **tachyzoite** immunodominant proteins revealed that they were identical to those of N. **hughesi** , a species described recently from a naturally infected horse. The isolate recovered from the naturally...

... horse in the present study (designated NA1) is thought to be an isolate of N. **hughesi** , although confirmation of this awaits additional molecular characterisation. These results provide some additional evidence that N. **hughesi** is a valid species and that Neospora infections in horses may occur in widely separated...

1/3,KWIC/26 (Item 1 from file: 35)
DIALOG(R)File 35:Dissertation Abs Online

01998773 ORDER NO: AADAA-I3120945
Evaluation of diagnostic tests and risk of exposure to the agents of equine **protozoal myeloencephalitis (EPM) in California**
Author: Duarte, Paulo de Camargo
Degree: Ph.D.
Year: 2003
Corporate Source/Institution: University of California, Davis (0029)
Source: VOLUME 65/02-B OF DISSERTATION ABSTRACTS INTERNATIONAL.
PAGE 601. 128 PAGES

...the accuracy of the IFAT in serum and cerebrospinal fluid (CSF) of

horses tested based on immunoblot analysis. The seroprevalence for S. **neurona** and N. **hughesi** antibodies was 0%. We concluded that these horses are either not routinely exposed to these...

... 3 years of age. This naive population of horses could be useful when evaluating S. **neurona** serodiagnostic tests or evaluating potential S. **neurona** **vaccines** since exposure risks to S. **neurona** and closely related parasites are negligible.

1/3,KWIC/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12685232 PMID: 10608440

Prevalence of antibodies to Neospora sp. in horses from Alabama and characterisation of an isolate recovered from a naturally infected horse [corrected]

Cheadle M A; Lindsay D S; Rowe S; Dykstra C C; Williams M A; Spencer J A; Toivio-Kinnucan M A; Lenz S D; Newton J C; Rolsma M D; Blagburn B L

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849, USA.

International journal for parasitology (ENGLAND) Oct 1999, 29 (10) p1537-43, ISSN 0020-7519 Journal Code: 0314024

Publishing Model Print; Erratum in Int J Parasitol 2000 Apr 24;30(5) 677

Document type: Case Reports; Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

...100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). **Tachyzoites** were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the horse with CNS disease. **Tachyzoites** reacted with known N. caninum-positive serum from horses, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or equine anti-Sarcocystis **neurona** serum. Ultrastructural features of **tachyzoites** and results of comparison of **tachyzoite** immunodominant proteins revealed that they were identical to those of N. **hughesi** , a species described recently from a naturally infected horse. The isolate recovered from the naturally...

... horse in the present study (designated NA1) is thought to be an isolate of N. **hughesi** , although confirmation of this awaits additional molecular characterisation. These results provide some additional evidence that N. **hughesi** is a valid species and that Neospora infections in horses may occur in widely separated...

1/3,KWIC/4 (Item 1 from file: 654)
DIALOG(R)File 654:US Pat.Full.

6090935
Derwent Accession: 2003-341035
UTILITY
Monoclonal antibodies to Sarcocystis neurona and uses therefor
Inventor: Marsh, Antoinette, Columbia, MO, US
Assignee: The Curators of the University of Missouri, (02), Columbia, MO,

DIALOG(R)File 155:MEDLINE(R)

11952574 PMID: 9234899

Micropreparative high resolution purification of proteins **by a combination of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, isoelectric focusing, and membrane blotting.**

Liang F T; Granstrom D E; Timoney J F; Shi Y F
Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington 40546, USA.

Analytical biochemistry (UNITED STATES) Jul 15 1997, 250 (1) p61-5, ISSN 0003-2697 Journal Code: 0370535
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

We report a simple, economical, and efficient protocol for **protein** purification from cells. First, **proteins** of cell lysates were separated by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted to **protein** -blotting membrane. The blots were stained with Coomassie blue or developed by immunoblotting to visualize specific **proteins** . The bands corresponding to those visible by immunoblotting were excised from the dye-stained blots and subjected to isoelectric focusing. The focused gel was stained with Coomassie blue. Finally, the stained bands were excised and subjected to another SDS-PAGE separation and electrotransferred back to **protein** -blotting membrane. At this stage, the purified **proteins** were suitable for microsequencing. We have tested the feasibility of this novel technique by purifying **proteins** with molecular weights ranging from 19 to 100 kDa from a lysate of **Sarcocystis neurona** , the etiologic agent of equine protozoal myeloencephalitis. The purity of **proteins** was demonstrated by reverse-phase high-performance liquid chromatography. Partial sequences of these purified **proteins** were obtained by N-terminal or digestive sequencing.

Tags: Research Support, Non-U.S. Gov't
Descriptors: ***Antigen** s, Protozoan--isolation and purification--IP; ***Proteins** --isolation and purification--IP; Animals; Chromatography, High Pressure Liquid; Electrophoresis, Polyacrylamide Gel; Immunoblotting; Isoelectric Focusing; Membranes, Artificial; **Sarcocystis** --immunology--IM
CAS Registry No.: 0 (Antigens, Protozoan); 0 (Proteins)
Record Date Created: 19970904
Record Date Completed: 19970904

5/9/13 (Item 13 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

11633290 PMID: 8944807

Neosporosis as a cause of equine protozoal myeloencephalitis.

Marsh A E; Barr B C; Madigan J; Lakritz J; Nordhausen R; Conrad P A
Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis 95616-8745, USA.

Journal of the American Veterinary Medical Association (UNITED STATES) Dec 1 1996, 209 (11) p1907-13, ISSN 0003-1488 Journal Code: 7503067
Publishing Model Print

5/9/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12685232 PMID: 10608440

Prevalence of antibodies to Neospora sp. in horses from Alabama and characterisation of an isolate recovered from a naturally infected horse [corrected]

Cheadle M A; Lindsay D S; Rowe S; Dykstra C C; Williams M A; Spencer J A; Toivio-Kinnucan M A; Lenz S D; Newton J C; Rolsma M D; Blagburn B L

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL 36849, USA.

International journal for parasitology (ENGLAND) Oct 1999, 29 (10) p1537-43, ISSN 0020-7519 Journal Code: 0314024

Publishing Model Print; Erratum in Int J Parasitol 2000 Apr 24;30(5) 677

Document type: Case Reports; Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

An IFAT was used to determine the prevalence of Neospora-specific IgG antibodies in serum from Alabama horses. Serum samples (n = 536) were from asymptomatic horses routinely submitted for equine infectious anaemia virus infection testing. We also subjected a 13-year-old horse with CNS disease to necropsy examination for isolation and in vitro cultivation of protozoal organisms. In antemortem tests, this horse was positive for antibodies to Neospora sp. in the IFAT and western immunoblot. Results of the prevalence survey indicated that IgG antibodies to Neospora were present in 62 (11.5%) of the 536 serum samples. Endpoint titres for the positive samples were 1:50 (35/6.5%), 1:100 (19/3.5%), 1:200 (7/1.3%) and 1:1600 (1/0.2%). Tachyzoites were first seen in cultured bovine turbinate cells 32 days after inoculation with spinal cord homogenates from the horse with CNS disease. Tachyzoites reacted with known N. caninum-positive serum from horses, cows, dogs and mice, but did not react with murine anti-Toxoplasma gondii or equine anti- **Sarcocystis neurona** serum. Ultrastructural features of tachyzoites and results of comparison of tachyzoite immunodominant **proteins** revealed that they were identical to those of N. hughesi, a species described recently from a naturally infected horse. The isolate recovered from the naturally infected horse in the present study (designated NA1) is thought to be an isolate of N. hughesi, although confirmation of this awaits additional molecular characterisation. These results provide some additional evidence that N. hughesi is a valid species and that Neospora infections in horses may occur in widely separated geographic regions of the United States.

Tags: Female; Research Support, Non-U.S. Gov't

Descriptors: *Antibodies, Protozoan--blood--BL; *Coccidiosis--veterinary --VE; *Horse Diseases--epidemiology--EP; *Neospora--immunology--IM; *Neospora--isolation and purification--IP; Animals; Antibodies, Protozoan --immunology--IM; Cattle; Coccidiosis--epidemiology--EP; Coccidiosis --parasitology--PS; Dogs; Fluorescent Antibody Technique, Indirect; Horse Diseases--parasitology--PS; Horses; Mice; Myelitis--parasitology--PS; Myelitis--veterinary--VE; Neospora--ultrastructure--UL; Prevalence; Spinal Cord--parasitology--PS

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20000120

Record Date Completed: 20000120

5/9/12 (Item 12 from file: 155)

12758328 PMID: 10690772

Improvement of western blot test specificity for detecting equine serum antibodies to Sarcocystis neurona .

Rossano M G; Mansfield L S; Kaneene J B; Murphy A J; Brown C M; Schott H C; Fox J C

Animal Health Diagnostic Laboratory, The Population Medicine Center, Michigan State University, East Lansing 48824, USA.

Journal of veterinary diagnostic investigation - official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc (UNITED STATES) Jan 2000, 12 (1) p28-32, ISSN 1040-6387

Journal Code: 9011490

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Equine protozoal myeloencephalitis (EPM) is a neurological disease of horses and ponies caused by the apicomplexan protozoan parasite **Sarcocystis neurona** . The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and to assess the ability of bovine antibodies to **Sarcocystis** cruzi to act as a blocking agent to minimize false-positive results in the western blot test for S. **neurona** . **Sarcocystis neurona** merozoites harvested from equine dermal cell culture were heat denatured, and the **proteins** were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient gel. Separated **proteins** were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6 horses with S. **neurona** infections (confirmed by culture from neural tissue) and 57 horses without infections (horses from the Eastern Hemisphere, where S. **neurona** does not exist) were tested by Western blot. Horses from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-kD bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the equine samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16-kD bands ($P<0.001$, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking **proteins** not specific to S. **neurona** and using reactivity to the 30- and 16-kD bands as the criterion for a positive test.

Tags: Research Support, Non-U.S. Gov't

Descriptors: *Antibodies, Protozoan--analysis--AN; *Cattle Diseases --parasitology--PS; *Encephalomyelitis, Equine--virology--VI; * **Sarcocystis** --immunology--IM; *Sarcocystosis--veterinary--VE; Animals; Blotting, Western--standards--ST; Cattle; Cattle Diseases--genetics--GE; Cattle Diseases--immunology--IM; Encephalomyelitis, Equine--genetics--GE; Encephalomyelitis, Equine--immunology--IM; **Sarcocystis** --genetics--GE; Sarcocystosis--genetics--GE; Sarcocystosis--immunology--IM; Sensitivity and Specificity

CAS Registry No.: 0 (Antibodies, Protozoan)

Record Date Created: 20000321

Record Date Completed: 20000321

Document type: Case Reports; Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

Neosporosis was diagnosed in an 11-year-old Quarter Horse gelding with clinical signs and diagnostic test results compatible with equine protozoal myeloencephalitis (EPM). Presumptive postmortem diagnosis of EPM attributable to **Sarcocystis neurona** infection is generally made on the basis of detecting an antibody titer to S **neurona** in the CSF or characteristic histologic lesions, even when parasites have not been specifically identified. Neosporosis was confirmed in the horse described here by use of immunohistochemical examination, in vitro culturing, and ultrastructural and molecular characterization of parasites from infected tissues. Antibody testing of serum and CSF samples indicated that Neospora-specific anti-bodies can react with S **neurona proteins** on western blot analysis. The confirmation that neosporosis in horses can mimic EPM emphasizes the need to broaden the etiologic definition of EPM beyond infections exclusively attributable to S **neurona** .

Tags: Male; Research Support, Non-U.S. Gov't

Descriptors: *Coccidiosis--veterinary--VE; *Encephalomyelitis--veterinary --VE; *Horse Diseases--parasitology--PS; *Neospora --isolation and purification--IP; Animals; Antibodies, Protozoan--cerebrospinal fluid--CF; Antibodies, Protozoan--immunology--IM; **Antigens** , Protozoan--analysis--AN ; Coccidiosis--parasitology--PS; Encephalomyelitis--parasitology--PS; Horses; Immunohistochemistry; Neospora--immunology--IM; Neospora --ultrastructure--UL; Spinal Cord--parasitology--PS; Spinal Cord --ultrastructure--UL

CAS Registry No.: 0 (Antibodies, Protozoan); 0 (Antigens, Protozoan)
Record Date Created: 19970130
Record Date Completed: 19970130

5/9/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10136275 PMID: 8466988

Equine protozoal myeloencephalitis: antigen **analysis of cultured** Sarcocystis neurona **merozoites.**

Granstrom D E; Dubey J P; Davis S W; Fayer R; Fox J C; Poonacha K B; Giles R C; Comer P F

Department of Veterinary Science, University of Kentucky, Lexington 40546-0099.

Journal of veterinary diagnostic investigation - official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc (UNITED STATES) Jan 1993, 5 (1) p88-90, ISSN 1040-6387
Journal Code: 9011490
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Languages: ENGLISH
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Subfile: INDEX MEDICUS

Antigens of cultured **Sarcocystis neurona** merozoites were examined using immunoblot analysis. Blotted **proteins** were probed with S. cruzi, S. muris, and S. **neurona** antisera produced in rabbits, S. fayeri (pre- and post-infection) and S. **neurona** (pre- and post-inoculation) sera produced

in horses, immune sera from 7 histologically confirmed cases of equine protozoal myeloencephalitis (EPM), and pre-suckle serum from a newborn foal. Eight **proteins** , 70, 24, 23.5, 22.5, 13, 11, 10.5, and 10 Kd, were detected only by S. **neurona** antiserum and/or immune serum from EPM-affected horses. Equine sera were titered by the indirect immunofluorescent antibody (IFA) method using air-dried, cultured S. **neurona** merozoites. Anti- **Sarcocystis** IFA titers were found in horses with or without EPM. Serum titers did not correspond to the number of specific bands recognized on immunoblots.

Tags: Research Support, Non-U.S. Gov't

Descriptors: ***Antigen** s, Protozoan--analysis--AN; *Horse Diseases; ***Sarcocystis** --immunology--IM; *Sarcocystosis--veterinary--VE; Animals; **Antigens** , Protozoan--isolation and purification--IP; Cattle; Cells, Cultured; Electrophoresis, Polyacrylamide Gel; Horses; Immunoblotting; Molecular Weight; **Sarcocystis** --isolation and purification--IP

CAS Registry No.: 0 (Antigens, Protozoan)

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DIALOG(R)File 155:MEDLINE(R)

09863907 PMID: 1644935

A five year (1985-1989) retrospective study of equine neurological diseases with special reference to rabies.

Hamir A N; Moser G; Rupprecht C E

Laboratory of Large Animal Pathology, University of Pennsylvania, New Bolton Center, Kennett Square 19348.

Journal of comparative pathology (ENGLAND) May 1992, 106 (4) p411-21 , ISSN 0021-9975 Journal Code: 0102444

Contract/Grant No.: AI-09206-16; AI; NIAID

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

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A retrospective study of horses necropsied between 1985 and 1989 at a diagnostic laboratory of a veterinary school in North America is documented. In this investigation over 20 per cent of the horses had clinical neurological signs. Equine protozoal myeloencephalitis (caused by **Sarcocystis neurona**) and cervical stenotic myelopathy (wobbler syndrome) were the most common of these disorders. The veterinary school is located in the midst of a raccoon rabies enzootic area. However, only four cases of equine rabies were diagnosed during the 5-year study. The gross microscopical and immunohistochemical findings from these rabies-positive horses are documented. Immunoperoxidase tests for detection of rabies **antigen** in another 35 horses with non-specific encephalitis/encephalopathy did not reveal any positive cases. Based on this investigation, it appears that immunoperoxidase is a valid method for diagnosis of rabies when fresh tissues are not available for the fluorescent antibody test. It is also concluded that no cases of equine rabies were overlooked by the diagnostic laboratory during the period under investigation.

Tags: Female; Male; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Descriptors: *Horse Diseases--pathology--PA; *Nervous System Diseases --veterinary--VE; *Rabies--veterinary--VE; Animals; Brain--microbiology--MI ; Brain--pathology--PA; Diagnosis, Differential; Horse Diseases --epidemiology--EP; Horses; Immunoenzyme Techniques; Nervous System Diseases--complications--CO; Nervous System Diseases--epidemiology--EP; Nervous System Diseases--pathology--PA; Pennsylvania--epidemiology--EP; Rabies--complications--CO; Rabies--epidemiology--EP; Rabies--pathology --PA; Retrospective Studies; Spinal Cord--microbiology--MI; Spinal Cord --pathology--PA

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12758328 PMID: 10690772

Improvement of western blot test specificity for detecting equine serum antibodies to Sarcocystis neurona .

Rossano M G; Mansfield L S; Kaneene J B; Murphy A J; Brown C M; Schott H C; Fox J C

Animal Health Diagnostic Laboratory, The Population Medicine Center, Michigan State University, East Lansing 48824, USA.

Journal of veterinary diagnostic investigation - official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc (UNITED STATES) Jan 2000, 12 (1) p28-32, ISSN 1040-6387

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Publishing Model Print

Document type: Journal Article

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Subfile: INDEX MEDICUS

Equine protozoal myeloencephalitis (EPM) is a neurological disease of horses and ponies caused by the apicomplexan protozoan parasite **Sarcocystis neurona** . The purposes of this study were to develop the most stringent criteria possible for a positive test result, to estimate the sensitivity and specificity of the EPM Western blot antibody test, and to assess the ability of bovine antibodies to **Sarcocystis** cruzi to act as a blocking agent to minimize false-positive results in the western blot test for S. **neurona** . **Sarcocystis neurona** merozoites harvested from equine dermal cell culture were heat denatured, and the **proteins** were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12-20% linear gradient gel. Separated **proteins** were electrophoretically transferred to polyvinylidene fluoride membranes and blocked in 1% bovine serum albumin and 0.5% Tween-Tris-buffered saline. Serum samples from 6 horses with S. **neurona** infections (confirmed by culture from neural tissue) and 57 horses without infections (horses from the Eastern Hemisphere, where S. **neurona** does not exist) were tested by Western blot. Horses from both groups had reactivity to the 62-, 30-, 16-, 13-, 11-, 10.5-, and 10-kD bands. Testing was repeated with another step. Blots were treated with bovine S. cruzi antibodies prior to loading the equine samples. After this modification of the Western blot test, positive infection status was significantly associated with reactivity to the 30- and 16-kD bands ($P<0.001$, Fisher's exact test). The S. cruzi antibody-blocked Western blot had a sample sensitivity of 100% and sample specificity of 98%. It is concluded that the specificity of the Western blot test is improved by blocking **proteins** not specific to S. **neurona** and using reactivity to the 30- and 16-kD bands as the criterion for a positive test.

Tags: Research Support, Non-U.S. Gov't

137151587 CA: 137(11)151587v PATENT

Use of SAG-1 gene of Sarcocystis neurona for diagnostic tests and vaccines for equine protozoal myeloencephalitis

INVENTOR(AUTHOR): Dame, John B.; Ellison, Siobhan P.; Yowell, Charles A.

LOCATION: USA

PATENT: U.S. Pat. Appl. Publ. ; US 20020115828 A1 DATE: 20020822

APPLICATION: US 962993 (20010924) *US PV234676 (20000922)

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0736690

AN ANTIGEN **TEST TO DETECT** EQUINE **PROTOZOAL MYELOENCEPHALITIS IN HORSE SERUM AND CEREBROSPINAL FLUID**

TEST D' ANTIGENES **POUR LA DETECTION DE MYELOENCEPHALITE PROTOZOAIRE** EQUINE **DANS LE SERUM ET LE LIQUIDE CEPHALORACHIDIEN EQUINS**

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Priority Application: US 99120831 19990219; US 99152193 19990902

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TREATMENT OF EQUINE **PROTOZOAL MYELOENCEPHALITIS**

TRAITEMENT DE LA MYELOENCEPHALITE PROTOZOAIRE EQUINE

Patent Applicant/Assignee:
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FENGER Clara K,

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IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL
PT RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN YU GH KE LS MW SD SZ UG
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LIVE ANTENUATED PARASITE VACCINE

VACCIN DE PARASITE VIVANT ATTENUE

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(EP) AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT RO SE SI SK TR
(OA) BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
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